Despite recognition of the possible role of biological nitrogen removal (BNR) processes in nitrous oxide (N$_2$O) emission, a measured database of N$_2$O emissions from these processes at the national scale does not currently exist. This study focused on the quantification of N$_2$O emissions at 12 wastewater treatment plants (WWTPs) across the United States using a newly developed U.S. Environmental Protection Agency (USEPA) reviewed protocol. A high degree of variability in field-scale measurements of N$_2$O was observed, both across the WWTPs sampled and within each WWTP. Additionally, aerobic zones, which have hitherto not been considered in the USEPA approach of estimating N$_2$O emissions, generally contributed more to N$_2$O fluxes than anoxic zones from BNR reactors. These results severely qualify the conventional use of a single emission factor to “estimate” N$_2$O emissions from BNR processes, solely by virtue of denitrification. Upon subjecting the nationwide data set to multivariate regression data mining, high nitrite, ammonium, and dissolved oxygen concentrations were positively correlated with N$_2$O emissions from aerobic zones of activated sludge reactors. On the other hand, high nitrite and dissolved oxygen concentrations were positively correlated with N$_2$O emissions from anoxic zones. Based on these results, it can be argued that activated sludge processes that minimize transient or permanent build up of ammonium or nitrite, especially in the presence of dissolved oxygen, are expected to have low N$_2$O emissions.

Introduction

Increasingly tighter regulations on total nitrogen removal from wastewater streams have spurred a global effort to develop and implement several cost-effective engineered biological nitrogen removal (BNR) strategies at point sources of nitrogen pollution such as wastewater treatment plants. Based on recent field-scale measurements, selected engineered BNR facilities, while effective to varying degrees in reducing aqueous nitrogen pollution could emit up to 7% of the influent nitrogen load as gaseous nitrous oxide (N$_2$O) and nitric oxide (NO), as described for the nitrifying B-stage of the Dokhaven-Sluisjesdijk WWTP in The Netherlands (1). Such emissions are deleterious to the environment. The greenhouse equivalence of N$_2$O is about three hundred times that of carbon dioxide and both N$_2$O and NO contribute to depletion of the ozone layer (2). Although, from a fundamental perspective, N$_2$O and NO are known intermediates in denitrification (3, 4) and nitrification (5–9), the net contribution of BNR processes to N$_2$O emissions from wastewater treatment has only recently been acknowledged (10). Based on the latest U.S. Environmental Protection Agency (USEPA) report on sources and sinks of N$_2$O from wastewater treatment operations, denitrification in anoxic zones is implicated as the dominant source of N$_2$O from BNR activated sludge reactors (10). However, nitrification could also play a role in N$_2$O generation and emission from WWTPs, especially under cycling between anoxic and aerobic conditions (1), as is common in BNR reactors.

Therefore, N$_2$O fluxes from aerobic zones of WWTPs might also need to be included in N$_2$O emissions inventories. At this point, while there is considerable debate and interest on the “potential” of constituent BNR processes for N$_2$O generation and emission, there are few reports that systematically quantify such emissions from full-scale BNR operations (1, 11–15). Of these, only one has been conducted in the United States and focuses on a single non-BNR WWTP in New Hampshire (11). The remaining full-scale studies have mostly been conducted in Europe and have employed different methods for measuring N$_2$O emissions including the use of grab samples (1, 11, 13–15) or online measurements (1, 12). Given the broad diversity of BNR and non-BNR configurations that exist in the United States, a more detailed N$_2$O emissions database of WWTPs is needed, specifically obtained using a consistent protocol.

The principal motivation of conducting such a detailed monitoring campaign is the limitation in the currently used method to “estimate” the N$_2$O emissions of WWTPs. The current method is based on emission factor values of 3.2 g N$_2$O/population equivalents (PE)/year from non-BNR operations and 7.0 g N$_2$O/PE/year for BNR operations (10, 11). Based on these two emission factors and an additional factor (0.5% of wastewater effluent nitrogen emitted as N$_2$O), domestic wastewater treatment plants contribute approximately 3% to the total CO$_2$ footprint of waste management operations and 1.6% to total worldwide N$_2$O emissions (10). However, both emission factors are based on a limited data set and may not be broadly representative.

Based on these outstanding gaps of knowledge, the specific objectives of this study were to 1. Develop a database of N$_2$O emissions from different activated sludge process configurations using a standard protocol, and 2. Identify key factors that are correlated with N$_2$O emissions from activated sludge.

Materials and Methods

Development and Application of a Protocol to Measure N$_2$O Fluxes From BNR Processes. The overall measurement campaign of this study was conducted using a recently developed USEPA reviewed protocol and quality assurance project plan for measuring liquid and gas phase N$_2$O concentrations and their fluxes from activated sludge tanks (16). Commercially available replicas of the USEPA surface emission isolation flux chamber (SEIFC) with a circular cross-sectional area of 0.13 m$^2$ were used to measure gaseous N$_2$O Fluxes From BNR Processes.
was calculated by multiplying the mass flux (J_{TKN} \text{ loading (mass/mass)}) or daily TKN load processed WWTP normalized to the daily influent total Kjeldahl nitrogen proportion of N\textsubscript{2}O emitted from each WWTP was computed Q_{\text{emission,m}} \text{ m}^3/\text{min} in aerated and anoxic zones was measured approximately five times a day using a modification of ASTM D1946. Briefly, a tracer gas consisting of 100 000 ppmv of helium (He) was introduced into the SEIFC at a known flow rate. He concentrations in the off-gas from the SEIFC were measured using Standard Methods (17) directly next to the SEIFC location at the same time and frequency.

Advective flow rate of headspace gas through the SEIFC (Q_{\text{emission}} \text{ m}^3/\text{min}) in aerated and anoxic zones was measured approximately five times a day using a modification of ASTM D1946. Briefly, a tracer gas consisting of 100 000 ppmv of helium (He) was introduced into the SEIFC at a known flow rate. He concentrations in the off-gas from the SEIFC were measured using Standard Methods (17) directly next to the SEIFC location at the same time and frequency.

### Calculation of N\textsubscript{2}O Emission Factors

N\textsubscript{2}O emission factors were computed by normalizing the total reactor N\textsubscript{2}O mass flux to the unit population equivalent flow rate (100 gal/PE/day) and were expressed in units consistent with the USEPA inventory report (g N\textsubscript{2}O/PE/year) (10). For aerobic zones, the helium-based advective gas-flow data were correlated to plant-recorded airflow rates for any given zone via linear regression and used to calculate diurnal N\textsubscript{2}O emission factors. For anoxic (nonaerated) zones lacking associated plant airflow data, the average of the experimentally obtained helium-based gas flow rates was used to calculate diurnal N\textsubscript{2}O emission factors.

### Operating Characteristics of Wastewater Treatment Processes Sampled

The 12 WWTPs at which N\textsubscript{2}O emissions were measured employed a variety of BNR and non-BNR process configurations (Table 1). Although the specific identities of the WWTPs cannot be described, they were broadly distributed around the Northeast (4), Mid-Atlantic (2), Midwest (2) and Southwest (4) regions of the United States. Additionally, 7 out of the 12 processes were sampled at minimum and maximum annual wastewater temperatures to examine seasonal temperature impacts on N\textsubscript{2}O generation and emission.

### Correlation Between WWTP Operating Conditions and N\textsubscript{2}O Emissions

Possible links between WWTP operating conditions and N\textsubscript{2}O emission fluxes were examined via multivariate linear regression modeling using SAS (Cary, NC). For aerobic zones, N\textsubscript{2}O fluxes were correlated with sampling location-specific pH, activated sludge mixed-liquid temperature, DO, NH\textsubscript{4}\textsuperscript{+}-N, and NO\textsubscript{2}-N concentrations and interactive combinations thereof. For anoxic zones, N\textsubscript{2}O fluxes were correlated with sampling location-specific sCOD, pH, activated sludge mixed-liquid temperature, DO, NO\textsubscript{2}-N, and NO\textsubscript{3}-N concentrations and interactive combinations thereof. Assumptions of state variable normality and equal variance were evaluated using error residual and covariance plots (not shown). The variables not normally distributed were log-transformed, verified for normality and equal variance and subsequently used for regression modeling. Time points where all state variables had not been measured simultaneously were not included in regression analysis.
Results and Discussion

N$_2$O Emission Fluxes from Activated Sludge Processes. A wide range of N$_2$O emissions was measured across the 12 WWTPs operated at different temperatures, configurations and influent TKN loads (Table I). On average, N$_2$O emission fractions varied from 0.01 to 1.8% or 0.01 to 3.3%, when normalized to influent TKN load or influent TKN load processed, respectively. These emission fractions were on the lower end of the range reported by previous studies, which varied between 0 and 15% of influent TKN load (1, 11–15).

In general, N$_2$O emissions in aerated zones were higher than those in anoxic zones (Figure 1). Therefore, the currently held premise that N$_2$O emissions from WWTPs mostly occur in the anoxic zones (10) is not accurate. Higher emissions from aerobic zones can be attributed to three possible phenomena: increased air-stripping of N$_2$O, N$_2$O production due to oxygen inhibited denitrification or N$_2$O production by nitrification during changes in aeration. While it was not possible to infer the specific contribution of these phenomena to overall emissions from our data set, attempts were indeed made to identify links between principal operating parameters and measured emissions (described in the modeling section). Several WWTPs did not have dedicated anoxic zones (Figure 1), although transient DO limiting or anoxic conditions were indeed observed in them, for instance in the plug-flow or oxidation-ditch processes (shown in the Supporting Information (SI)).

Computed flow-normalized emission factors also varied in a wide range (Table I), and for several processes, were statistically higher ($\alpha = 0.05$) than currently used values of 3.2 g N$_2$O/PE/yr (non-BNR processes (11)) or 7.0 g N$_2$O/PE/yr (BNR processes (10)). Emission factors from the separate-stage BNR and oxidation ditch process were statistically lower, ($\alpha = 0.05$) and those from the two MLE processes were statistically not dissimilar, ($\alpha = 0.05$) relative to the current estimates (10). The centrate treatment process received the centrifuge supernatant of the anaerobic digestion recycle stream and was operated to partially convert the influent NH$_4^+$-N load to NO$_2^-$-N. Such operation is intended to reduce oxygen requirements for nitrification and organic carbon requirements for subsequent denitrification (as a variant of the single reactor high-activity ammonia removal over nitrite, SHARON process (18)). Emission factors for the

FIGURE 1. N$_2$O emissions from aerobic and anoxic zones in different WWTPs measured at high (A) and low (B) temperatures. Specific temperatures described in Table I. Step-feed BNR 3 is not included since the emissions from the covered aerobic and anoxic zones could not be distinctly measured.
separate centrate treatment process were not computed because centrate streams have disproportionately low flow rates compared to their TKN concentrations (19), which lead to incomparably high emission factor values. A high degree of diurnal variability in emission factors from the overall processes sampled was also observed (shown for three select processes in Figure 2) and could be linked to diurnal variations in influent N-loading (data not shown) as reported previously (20). Based on the observed variability either diurnally or across the range of WWTPs sampled, the use of a “single” universal emission factor to calculate N₂O emissions from all wastewater treatment processes is inadequate and strongly discouraged. Further, due to significant differences in local or global wastewater composition (19), the use of flow...
within activated sludge flocs even under "bulk" aerobic N-cycling in aerobic zones, the possibility of denitrification relation of NH4\(^+\) nitrification (interaction effect), and NH4\(^+\) and NO2\(^-\) concentrations (interactive effect, Table II). From a fundamental perspective, nitrite is one of the most important factors contributing to N2O production from nitrification for different ammonia oxidizing bacteria including Nitrosomonas europaea (21–23) and several strains of Nitrosospira spp. (24), which are expected to be more abundant in activated sludge (25). Thus, correlation of NO2\(^-\) concentrations with aerobic zone N2O emissions is consistent with basic biochemical principles. Although it was previously proposed that low DO concentrations were directly responsible for nitrification-related N2O emissions (26), recent results suggest that N2O is generated by recovery from low DO conditions rather than imposition thereof (27). In fact, abrupt increases in DO concentrations in the presence of NH4\(^+\) can also lead to transient accumulation of NO2\(^-\), which can in turn result in NO and N2O generation from nitrification (27, 28). The magnitude of nitrification driven N2O emissions also depends directly on NH4\(^+\)-N concentrations (27). Therefore, the positive correlation of NH4\(^+\)-N and DO concentrations individually with N2O emissions in the aerobic zones is in congruence with this new understanding of aerobic N2O production from nitrification (27). The positive interactive correlation between NH4\(^+\) and NO2\(^-\) concentrations and N2O emissions suggests high N2O emissions from aerobic zone locations, with simultaneously high concentrations of both NH4\(^+\) and NO2\(^-\). This interactive correlation also points to N2O generation mechanisms from nitrification, wherein NH4\(^+\) and NO2\(^-\) can act as cosubstrates, (as described for Nitrosomonas europaea (21–23) and Nitrosospira spp. (24)).

It should be noted that while nitrification dominates N-cycling in aerobic zones, the possibility of denitrification within activated sludge flocs even under “bulk” aerobic conditions cannot be discounted (29). Interestingly, as with nitrification, denitrification driven N2O emissions are also caused by high DO and NO2\(^-\)-N concentrations (4, 30–33), among other factors. Thus, aerobic zone N2O emissions might be linked to both nitrification and denitrification, although it is not possible to differentiate between the two using bulk headspace measurements alone.

Anoxic Zones. Several factors have been implicated in N2O emissions from denitrifying bioreactors, such as low pH (34), short solids retention time (35), organic carbon limitation (35–37), DO inhibition (31, 33), and NO2\(^-\)-N inhibition (4, 30, 32). Inhibition of denitrification by high DO concentrations could also lead to NO2\(^-\)-N build-up, indirectly leading to N2O emissions (35, 38). Thus, the positive correlation of DO and NO2\(^-\)-N concentrations with N2O emissions (Table III) is consistent with known mechanisms of denitrification-related N2O production. Soluble COD concentrations in anoxic zones were mostly nonlimiting (29) (provided in the SI) and expectedly did not correlate with N2O emissions.

The regression model did not correlate temperature and N2O fluxes in either aerobic or anoxic zones (Tables II–III). A possible reason could be that N2O fluxes are indirectly governed by temperature through manifestation in NH4\(^+\)-N, NO2\(^-\)-N, or DO concentrations, as described above. For instance, in a process not designed or under-designed for nitrification, high NH4\(^+\)-concentrations are more distributed over the entire bioreactor volume during low temperatures (due to lower nitrification kinetics and lower nitrifying bacteria concentrations). Additionally, the lower temperatures also could potentially increase the solubility of oxygen in the mixed-liquor (39). Given that nonlimiting NH4\(^+\)-N and DO concentrations lead to N2O (27) (and results from correlation analysis in Table II), N2O emissions are expected to be higher in such systems at low temperature. In contrast, for systems that are designed to completely nitrify throughout the year (and have limiting reactor and effluent NH4\(^+\)-concentrations), the emissions of N2O are expected to be higher during higher temperature (due to higher overall kinetics of both oxidative and reductive nitrogen transformations). Notwithstanding the above argument, the actual configuration of the bioreactor also contributes to N2O emissions by virtue of localized concentrations and rate-determining regimes. Thus, it might be more illustrative to correlate N2O emissions with localized N-species and DO concentrations rather than with operating temperature in general.

While several factors relevant to activated sludge processes including NH4\(^+\), NO2\(^-\), DO, temperature, COD limitation can all lead to N2O emissions, it may be difficult to discern their specific contributions from highly noisy full-scale data from multiple plants, each operated under different modes and temperatures. Therefore, it is noteworthy that N2O emissions in aerobic and anoxic zones could be correlated with select specific factors and explained via mechanistic arguments. Further, all model parameter estimates except the intercept of the anoxic zone were statistically valid at the α = 0.05 level (Tables II–III). Nevertheless, it should be noted that the developed model is not a conclusive mechanistic descriptor of causative biological processes contributing to N2O emissions from activated sludge. Caution is also advised with respect to predicting emissions on the basis of process configuration alone. The factors correlating with N2O emissions, (NH4\(^+\)-N, NO2\(^-\)-N, and DO concentrations) are inherently linked with process parameters such as TKN loadings, solids residence time (SRT) and wastewater composition. Therefore, the propensity for N2O emissions of any given WWTP configuration can only be evaluated within the joint framework of its process operation and performance characteristics.

**TABLE II. Factors Correlating With N2O Emission Fluxes (g N2O–N/day) from Aerobic Zones**

| variable | parameter estimate | standard error | t value | Pr > |t |
|----------|------------------|---------------|---------|-------|
| intercept | 6.1 | 0.48 | 13 | 0.00 |
| log (ammonium) | 1.0 | 0.25 | 4.1 | 0.00 |
| log (nitrate) | 0.60 | 0.16 | 3.7 | 0.00 |
| log (DO) | 0.59 | 0.18 | 3.2 | 0.00 |
| log (ammonium)− log(nitrate) | 0.18 | 0.08 | 2.3 | 0.03 |

**TABLE III. Factors Correlating with N2O Emission Fluxes (g N2O–N/day) from Anoxic Zones**

| variable | parameter estimate | standard error | t value | Pr > |t |
|----------|------------------|---------------|---------|-------|
| intercept | −1.2 | 0.89 | −1.3 | 0.21 |
| log(DO)− log(nitrite) | 0.67 | 0.25 | 2.7 | 0.01 |

**TABLE IV. Logarithmic Relationships Between N2O Emission Rates (g N2O–N/day) and N2O Emission Factors (PSU) for Aerobic Zones**

<table>
<thead>
<tr>
<th>log (ammonium)</th>
<th>log (nitrite)</th>
<th>log (DO)</th>
<th>log (ammonium)− log(nitrite)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.60</td>
<td>0.18</td>
<td>3.7</td>
<td>0.00</td>
</tr>
<tr>
<td>0.18</td>
<td>0.08</td>
<td>2.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**TABLE V. Logarithmic Relationships Between N2O Emission Rates (g N2O–N/day) and N2O Emission Factors (PSU) for Anoxic Zones**

<table>
<thead>
<tr>
<th>log(DO)</th>
<th>log(nitrite)</th>
<th>log(DO)− log(nitrite)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1.2</td>
<td>0.89</td>
<td>−1.3</td>
</tr>
<tr>
<td>0.67</td>
<td>0.25</td>
<td>2.7</td>
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</tbody>
</table>
N2O Emissions From WWTPs and Sustainable BNR Design and Operations. The sustainable management of nitrogenous pollution is one of the grand challenges of the National Academy of Engineering (http://www.engineeringchallenges.org/). Thus, from an engineering perspective, it would be beneficial to develop sound strategies for BNR design and operation that minimize both aqueous and gaseous nitrogen emissions. Based on this study, the measured emission factors from some BNR processes (Table I) were at least 1 order of magnitude higher than current estimates (10). However, given that the emission factors from other processes, were clearly lower than current emission factor estimates (Table I), the prospect of engineering based minimization N2O emissions from WWTPs appears to be feasible.

It should nevertheless be strongly emphasized that the results of this study do not implicate biological wastewater treatment and biological nutrient removal as prime contributors to atmospheric N2O fluxes. Even if the emission factors of all WWTPs were to hypothetically be universally an order of magnitude higher (which is highly unlikely, as shown in this study), domestic wastewater treatment would still contribute just 14% of the overall N2O emissions (using the same figures for N2O emissions from other sources, from (10)). Clearly, compromising domestic wastewater treatment and nutrient removal for the sake of reducing N2O emissions could have far more negative implications to water quality and human health.

More than a greenhouse or ozone depletion perspective, low N2O emissions from BNR processes can also be an indication of stable nitrification and denitrification (27, 40). Based on regression modeling results (Tables II–III), BNR processes that avoid high NH4+-N, NO2--N, and DO concentrations and transients thereof are expected to generate less N2O, from both nitrification and denitrification. High NH4+-N, DO and NO2--N concentrations are typically encountered in under-designed and overaerated nitrification reactors (29). Therefore, aerobic processes that avoid incomplete or intermittent nitrification and overaeration are expected to have lower N2O emissions. Additionally, processes that rely on more uniform spatial DO profiles to promote simultaneous nitrification and denitrification such as the oxidation ditch sampled herein, are likely to have low N2O emissions. Such processes avoid repeated switching between aerobic and anoxic conditions, which in conjunction with high NH4+-N and DO concentrations are a prime cause for nitrification driven N2O emissions (27). An additional strategy to minimize N2O emissions could be to minimize the peaking factor of influent nitrogen loading to activated sludge via flow equalization (20). While flow equalization of primary effluent may be difficult because of the high flow rates, it might be possible in select WWTPs to blend high nitrogen containing centrate with primary effluent to equalize influent nitrogen loads.

This study is the first of its kind in the United States and there are only two other similar studies being conducted, in The Netherlands (Prof. M.C.M. van Loosdrecht, Delft University of Technology, personal communication) and Australia (Prof. Z. Yuan, University of Queensland, personal communication). To facilitate a global monitoring effort, the protocol developed in this study has been shared upon request with additional groups in Spain, Portugal, Germany, and Belgium, which are just beginning similar monitoring programs. It is expected that broad application of the protocol will allow WWTPs to quantify their N2O emissions using a standard approach and to ultimately engineer environmentally sustainable BNR operating strategies and configurations.

Acknowledgments
This study is supported by the Water Environment Research Foundation. The monitoring campaigns will be continued over the next few years to broaden the sampling pool of WWTPs including those that will transition to BNR operations in the time being. As the monitoring progresses, updates to the emissions database will be presented on the website (www.columbia.edu/~kc2288/CCR) and in follow up publications.

Supporting Information Available
Process schematics of WWTP reactors and the process configuration table. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited


