

Revisiting the application of open-channel estimates of denitrification

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Abstract

Development of an open-channel method for measurement of denitrification, without the use of expensive isotopic tracers, has generated considerable interest among researchers attempting to quantify N loss from lotic systems. Membrane inlet mass spectrometry allows measurement of small changes in N₂ concentrations, facilitating calculation of whole reach denitrification rates using an N₂ mass balance corrected for gas exchange. The method has been applied successfully within numerous rivers ranging widely in size and denitrification rate. Previous model-based analyses suggest that the method can be applied in a broader suite of ecosystems, and specifically, that it is well suited to shallow streams where denitrification rates as low as 30–100 μmol N m⁻² h⁻¹ may be measurable. This coupled with increasing availability of necessary equipment, relatively low cost of measurements, and the ability to measure denitrification at environmentally relevant spatial scales suggests that broad adoption of the method is likely. In this paper, we revisit this model-based analysis using alternate models of gas exchange and demonstrate that benthic turbulence-induced gas exchange will restrict the suite of suitable study streams. Specifically, we note that within shallow streams and fast-flowing systems denitrification may be measurable only at moderate or high rates. To help facilitate further application of the method, we extend our discussion beyond site selection to discuss assumptions of the open-channel method, options for estimating the error in denitrification rates, and recommended practices for future studies.

The open-channel method allows the measurement of stream denitrification under natural conditions, without expensive ¹⁵N-tracers, with greater ease than traditional total nitrogen mass-balance techniques and without limitations associated with incubations and inhibitor-based methods (Laursen and Seitzinger 2002; Groffman et al. 2006; Table 1). The open-channel method applies a simple N₂ mass balance. N₂ is gained due to denitrification and groundwater inputs, and lost (or gained) via air-water gas exchange. Nitrogen fixation, anaerobic ammonium oxidation (anammox), N₂ loss to

groundwater and nitrous oxide (N₂O) production via denitrification are typically assumed to be negligible. Similar measurements based on excess N₂ have been applied in groundwater, riparian zones, lakes, and oceans (Groffman et al. 2006).

The open-channel method has been applied across a relatively broad suite of streams and rivers (Web Appendix 1—Table 1), and modifications to the method have allowed application in deep rivers and estuaries (Yan et al. 2004; Kana et al. 2006). Open-channel estimates have been compared with total nitrogen mass balance-based estimates of denitrification, yielding broadly similar results for the South Platte and Connecticut Rivers (Pribyl et al. 2005; Smith et al. 2008). If streams have low gas exchange coefficients and well-characterized groundwater N₂ inputs, the open-channel method may be preferred over mass balances due to easier implementation and reduced uncertainty (Pribyl et al. 2005), although the error associated with the method is sometimes high (Web Appendix 1—Table 1).

The open-channel method is analogous to oxygen mass balances used to determine rates of aquatic photosynthesis and res-

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Table 1. Major methods of measuring denitrification in streams.**Stream or reach-scale measurements**

Major advantages: These measurements are typically performed under in-stream conditions at relatively large spatial scales with direct relevance to broad scale N models.

Major limitations and assumptions: Cannot be directly replicated, therefore uncertainty estimates must be model-based. Tend to obscure importance of microscale processes.

<i>Open-channel N₂ accumulation</i>	<p><i>Method and general principle:</i> Accumulation of N₂ is measured over time, and model-based analyses are performed to partition biological from physical processes and estimate denitrification rates.</p> <p><i>Major advantages:</i> Where equipment is available, costs are relatively low. Environmental perturbation is minimal.</p> <p><i>Major limitations and assumptions:</i> Suite of suitable streams is restricted. Requires detailed modeling and careful measurements to partition physical and biological controls on N₂ accumulation. Measures “net denitrification.”</p> <p><i>Optimal conditions:</i> See Web Appendix 1—Table 2.</p>
<i>Natural abundance isotopic analysis of nitrate</i>	<p><i>Method and general principle:</i> Denitrification leads to ¹⁵N and ¹⁸O enrichment of the residual NO₃ pool. Measurements depend upon concurrent measurements of nitrate concentration and δ¹⁵N, as well as known fractionations (Kellman and Hillaire-Marcel 1998).</p> <p><i>Major advantages:</i> Represents in situ conditions (no environmental perturbations)</p> <p><i>Major limitations and assumptions:</i> High analytical costs. Careful characterization of all nitrate sources is required. Presence of multiple nitrate sources may confound results. Oxygen-exchange reactions may complicate interpretation of δ¹⁸O-NO₃. Ineffective if denitrification proceeds to completion. Magnitude of fractionation may vary.</p> <p><i>Optimal conditions:</i> Low groundwater inputs, low lateral inflows. Residual NO₃ pool (denitrification does not proceed to completion).</p>
<i>Mass balance</i>	<p><i>Method and general principle:</i> Denitrification is measured as the difference between N inputs and outputs (Sjodin et al. 1997)</p> <p><i>Major advantages:</i> Straightforward, widely used. Analytical equipment widely available.</p> <p><i>Major limitations and assumptions:</i> Unmeasured N inputs (N fixation, groundwater) and outputs (seasonal N uptake by autotrophs), transformations (mineralization, nitrification) and changes in storage will affect N balance and estimation of denitrification rates.</p> <p><i>Optimal conditions:</i> Systems where all parameters can be precisely constrained (see Groffman et al. 2006 for warnings regarding application of this method).</p>
<i>Whole-stream addition of ¹⁵N-enriched nitrate</i>	<p><i>Method and general principle:</i> ¹⁵N-enriched NO₃ is added to a stream. Denitrification is calculated using measurements of ¹⁵N-enriched N₂, corrected for gas exchange.</p> <p><i>Major advantages:</i> Allows denitrification measurement with minimal environmental perturbation. Even relatively low rates of denitrification can be measured.</p> <p><i>Major limitations & assumptions:</i> High cost of tracer and analyses. Excludes denitrification that is coupled to mineralization and nitrification. Surface loading does not allow mixing into deeper sediment layers and may underestimate denitrification (Mulholland et al. 2004). Nitrate addition may stimulate denitrification.</p> <p><i>Optimal conditions:</i> High cost of tracers restricts this approach to relatively low-flow systems with low-moderate background nitrate concentrations.</p>

piration, and as in oxygen mass balances, two different approaches may be applied. A two-station method follows a single parcel of water through time and space, and to date, has been the more common approach in open-channel denitrification measurements. Many studies extend beyond two stations into three or more stations, with time of travel and gas transfer known between each series of stations. This allows better spatial representation of denitrification rates and could also be used to assess error associated with the method at broader spacing of study stations. The one station, or diel approach (e.g., McCutchan et al. 2003) employs a time for space substitution. While the methods are con-

ceptually similar and mathematically equivalent, the assumptions inherent in each differ (see “Assessment and Discussion”).

The most significant limitation of the open-channel method relates to detection limits and uncertainty of the method. Whereas N₂ concentrations can be measured with high precision (coefficient of variation 0.5% for N₂, 0.05% for N₂:Ar) using membrane-inlet mass spectrometry (MIMS, Kana et al. 1994), our ability to measure denitrification rates using the open-channel method depends not only on instrumental precision and accuracy, but also on rates of N₂ loss and gain. Method uncertainty is dependent on a suite of parameters

Table 1. Continued**Patch-scale measurements in chambers**

Major advantages: These measurements are typically performed in flow-through chambers or static chambers of varying size. They allow relatively easy experimental manipulation, can be highly replicated, and allow isolated study of communities of interest.

Major limitations and assumptions: Scaling to reach or ecosystem levels can incorporate significant error (see Kellman 2004). Incubations can lead to changes in oxygen, carbon, and nitrogen concentrations, flow patterns, physical structure of substratum, boundary layer thickness, and microbial populations which may induce changes in denitrification rates.

Optimal conditions: Broadly applicable across most or all conditions.

N_2 or $N_2:Ar$	<p><i>Method and general principle:</i> Production of N_2 is measured in chambers, with N_2 accumulation representing “net denitrification.” (Denitrification minus nitrogen fixation). N_2 accumulation is often modeled based on measurements of $N_2:Ar$, and N_2 is calculated assuming Ar is at saturation.</p> <p><i>Major advantages:</i> Inexpensive.</p> <p><i>Major limitations & assumptions:</i> Can require long periods of incubation. Necessary equipment is only beginning to become widely available. Measures net, rather than gross denitrification. Water temperature must be carefully controlled to prevent misinterpretation of physical gas flux as denitrification or N_2 fixation.</p> <p><i>Optimal conditions:</i> Production of bubbles is extremely problematic. As a result, low rates of benthic ebullition, and low rates of photosynthetic O_2 production, are necessary.</p>
Acetylene inhibition	<p><i>Method and general principle:</i> Acetylene inhibits the reduction of N_2O to N_2 in the final step of denitrification. Denitrification is measured as the accumulation of N_2O.</p> <p><i>Major advantages:</i> Inexpensive, straightforward, and widely used in the past.</p> <p><i>Major limitations & assumptions:</i> Numerous analytical problems have been identified including: incomplete inhibition of the N_2O-N_2 step of denitrification (e.g., due to slow diffusion, sulphide interferences), inhibition of nitrification, (leading to underestimation of denitrification in systems with highly coupled nitrification-denitrification), and stimulation of denitrification via addition of a carbon substrate.</p> <p><i>Optimal conditions:</i> Can be applied in a broad suite of systems.</p>
^{15}N labeling	<p><i>Method and general principle:</i> ^{15}N labeled NO_3 is added to a chamber, and production of ^{15}N labeled gases is measured (Nielsen 1992).</p> <p><i>Major advantages:</i> Can measure gross denitrification (excludes N_2 fixation) and importance of water column NO_3 versus nitrification derived NO_3 as the substrate for denitrification.</p> <p><i>Major limitations and assumptions:</i> Nitrate pool must be evenly labeled. Nitrate addition can stimulate denitrification. There is potential for significant error in systems where nitrification and denitrification are tightly coupled, or where anammox is important (Thamdrup and Dalsgaard 2002).</p> <p><i>Assumptions:</i> Complete label mixing.</p> <p><i>Optimal conditions:</i> Homogenous substrates where assumption of complete label mixing is a reasonable assumption. See warnings in Groffman et al. (2006) regarding application in complex aquatic systems.</p>

including gas exchange rates (and scaling of these rates to the temperatures and gases of interest), gas solubility (therefore water temperature, pressure, and uncertainty in solubility equations), reach depth, N_2 concentrations in stream water and groundwater, and rates of groundwater gain and loss (see McCutchan et al. 2003; Laursen and Seitzinger 2005; Smith et al. 2008). The ratio of areal denitrification rates to overlying water volume affects the maximum rate of increase in N_2 concentrations. The rate of air-water gas exchange dictates how quickly excess N_2 is lost to the atmosphere. Groundwater inputs and outputs, nitrogen fixation, ebullition, diffusion, mixing, and temperature also affect N_2 accumulation and measurement of denitrification rates.

Laursen and Seitzinger (2005) performed a model-based analysis of factors affecting method sensitivity and detection limits.

Air-water gas exchange was estimated using a wind-based model of gas exchange, illustrating that the method is most sensitive at low wind speeds when gas exchange rates are low. They found that stream depth was a critical factor affecting the detection limit. At constant denitrification rates, N_2 accumulates more rapidly within shallow streams, contributing to an ability to measure lower rates of denitrification. Temperature also affected detection limits via effects on solubility and rates of gas transfer. This analysis focused on systems with wind-driven gas exchange. However, within many fluvial systems, and particularly in shallow systems, friction caused by water flow over the bottom substrate is a major cause of turbulent mixing and an important control on rates of gas transfer (Raymond and Cole 2001).

In the first part of this article, we revisit site-selection criteria for application of the open-channel method. Based on the

expectation that bottom turbulence is an important determinant of gas exchange in many small streams, we applied alternate models of water-air gas exchange and predicted that both stream depth and stream velocity would be important controls upon rates of gas exchange, N_2 accumulation, and as a result, the utility of the open-channel method. We anticipated that whereas dilution effects would be lower in shallow streams, gas exchange coefficients were likely to be higher, and shallow streams might not constitute ideal systems for application of this method. Because the open-channel method is extremely sensitive to rates of gas transfer, and existing models of water-air gas transfer incorporate significant error, measurements of denitrification require direct, concurrent measurements of gas exchange rates. However, our model analysis, in conjunction with the Laursen and Seitzinger (2005) analysis, will help researchers identify candidate streams for application of the open-channel method to measure denitrification. This is particularly important at preliminary stages of project planning and in the grant application and review process.

In the second part of this paper, best practices for implementation of the method are reviewed. We discuss assumptions of both the one-station and two-station approaches, and the major sources of uncertainty in open-channel measurements of denitrification. We review considerations for field and laboratory measurements and subsequent modeling of uncertainty to help guide researchers in the use of the open-channel method. Finally, we discuss alternative methods for measuring denitrification in systems where open-channel methods may not be appropriate, or where comparisons among methods are to be performed.

Materials and procedures

A model-based approach was applied to determine the suitability of shallow (≤ 1 m) streams for application of the open-channel method. Briefly, we modeled N_2 accumulation using a simple N_2 mass balance model using Matlab (Matlab R2008b 7.7.0, The MathWorks). N_2 accumulation was modeled within a parcel of water with a 1-min time step according to Eq. 1 (see below). The mathematical formulation of this model is identical for one-station and two-station approaches. $N_{2(t)}$ is the concentration of dinitrogen gas ($\mu\text{mol } N_2 \text{ L}^{-1}$) at time t ; $N_{2\text{sat}}$ is the saturation concentration of gas ($\mu\text{mol } N_2 \text{ L}^{-1}$). Denitrification is a calculation of the volumetric N_2 input resulting from denitrification ($\mu\text{mol } N_2 \text{ L}^{-1} \text{ min}^{-1}$). K is the gas transfer coefficient for N_2 (min^{-1}). The length of the time step (t) is 1 min.

***K* models**—Six alternative models of gas transfer coefficient (K) were incorporated into the N_2 model: three models based on wind-induced aeration (Wanninkhof 1992; Cole and Caraco 1998; Laursen and Seitzinger 2005) and three models where benthic turbulence-induced gas exchange is estimated based on

stream depth and velocity (O'Connor and Dobbins 1958; Churchill et al. 1962; Owens et al. 1964; see Web Appendix 2 for details including model formulae). Because no single model appears adequate for describing rates of gas transfer across the diverse conditions within streams (Melching and Flores 1999; Gualtieri et al. 2002), we apply this range of model outputs as an indicator of whether the open-channel method may be applicable in a single study stream. These equations were selected based on their limited data requirements and resulting usefulness in making first assessments of gas exchange; however, these are just a few examples of a vast literature describing rates of gas transfer. More complex models that incorporate factors such as slope and roughness may provide more accurate results in some systems. If the criterion of Schwarzenbach et al. (1993, see Web Appendix 2) is applied, the majority of our study scenarios (Fig. 1) reflect systems where benthic turbulence is likely to drive gas exchange. The only exception is at a depth of 1 m, and the minimum stream velocity tested of 0.02 m s^{-1} where wind models are expected to be more appropriate (see Web Appendix 2). Revisiting method sensitivity is clearly necessary as previous analyses (Laursen and Seitzinger 2005) were based on the assumption of wind-induced gas exchange. We provide the wind model analysis to highlight differences from the previous analysis. In subsequent discussion, we refer to all gas exchange models by using the name of only the first author.

Model-based analysis—The N_2 model was run for denitrification rates ranging from 100 to 2000 $\mu\text{mol } N \text{ m}^{-2} \text{ h}^{-1}$ (in 100 $\mu\text{mol } N \text{ m}^{-2} \text{ h}^{-1}$ increments) for each K model under a range of stream depths, velocities, and wind speeds (Fig. 1). We recorded the time after the model start (at saturation conditions) that N_2 accumulation due to denitrification could be distinguished reliably from background N_2 concentrations; that is, the time at which N_2 accumulation is greater than or equal to N_2 accumulation in the absence of denitrification plus an error term: $N_2 \text{ modeled}_{\text{denitrification} = x} > N_2 \text{ modeled}_{\text{denitrification} = 0} + \text{error term}$.

To facilitate comparison with the work of Laursen and Seitzinger (2005), we use an error term of $1 \mu\text{mol } N_2 \text{ L}^{-1}$. This figure is based on analytical error of 0.22 to $0.41 \mu\text{mol } N_2 \text{ L}^{-1}$. Whereas this figure reflects the precision of the method (i.e., reproducibility of multiple replicates), method accuracy (i.e., difference from the true value) can be somewhat poorer. Analyses of Kana et al. (1994) indicate that differences between measured values and expected values across a range of concentrations from 326 to $473 \mu\text{mol } N_2 \text{ L}^{-1}$ were typically between 0.24% and 1.65%, depending on temperature and whether N_2 , or $N_2:\text{Ar}$ was considered. Accuracy of analyses is likely to vary among labs, and over time, depending on factors such as instrument stability. For comparative purposes, we reran the same model analysis, assuming a considerably higher error term of $4 \mu\text{mol } N_2 \text{ L}^{-1}$.

$$N_{2(t+1)} = N_{2(t)} \frac{\mu\text{mol}}{\text{L}} + \left(\text{denitrification} \frac{\mu\text{mol } N_2}{\text{m}^2 \times \text{h}} \times \frac{1 \text{ m}^3}{1000 \text{ L}} \times \frac{1}{\text{depth (m)}} \times \frac{1 \text{ h}}{60 \text{ min}} \right) \times t_{(\text{min})} - \left((N_{2(t)} \frac{\mu\text{mol}}{\text{L}} - N_{2\text{sat}} \frac{\mu\text{mol}}{\text{L}}) \times K \frac{1}{\text{min}} \right) \times t_{(\text{min})} \quad (1)$$

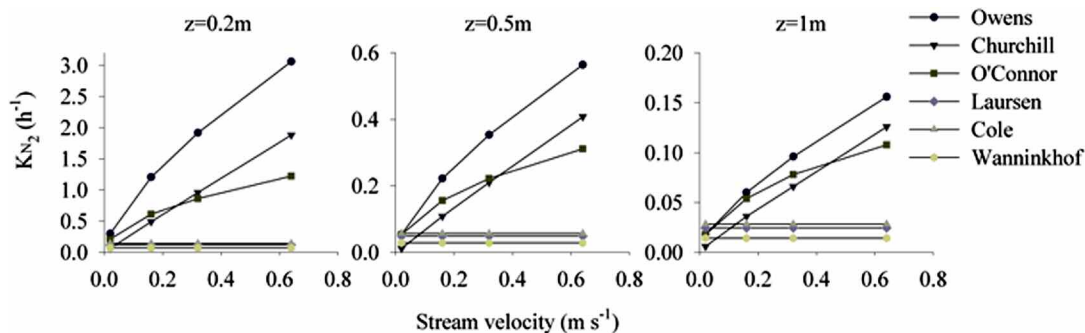


Fig. 1. K values resulting from 6 reaeration models under simulation conditions. These K values are used to generate results in Figs. 2 and 3. The first three models are indicative of benthic turbulence induced gas exchange. The latter three models estimate gas exchange driven by wind speeds of 2 m s^{-1} . See Web Appendix 2 for further details.

The model was run from saturation conditions using a 1 min time step for up to 12 d. We plot the time after model start that denitrification becomes measurable based on these two error terms in Figs. 2 and 3. We also note (at the top of the figures) scenarios where even after 12 d denitrification rates were not measurable. All model runs were performed at a temperature of 20°C , pressure of 1008.3 hPa, and wind speed of 2 m s^{-1} (wind speeds are at 10 m height). These conditions were selected to facilitate easy comparison with the model analyses of Laursen and Seitzinger (2005). If temperature and pressure are fixed, they have minor effects on model results, while wind speed can have a more important influence (Laursen and Seitzinger 2005). In studies that have applied the open-channel method to date, nitrogen fixation, anammox, loss of N_2 in bubbles, and loss of N_2 to groundwater have been assumed to be negligible and are excluded from this model. Likewise, N_2 gain from groundwater is also excluded from the model-based analysis. However, the importance of these error terms is discussed.

This analysis only determines that biotic N_2 production can be distinguished from physical N_2 fluxes, and does not constrain the uncertainty in the measurements. Given that measurement uncertainty can be quite significant (Web Appendix 1—Table 1), this is a broad first approximation of suitable streams.

Assessment and discussion

Site selection based on modeled gas transfer—The initial time point in our model-based analysis is at saturation conditions. Within shallow systems with high gas transfer and a high degree of diel temperature variation, measured N_2 concentrations may bisect saturation concentrations twice per day, suggesting that researchers studying systems such as these would be restricted to sites that show measurable N_2 accumulation in time periods $< 8\text{--}12 \text{ h}$. In contrast, in shallow systems with low gas exchange rates and sufficiently high denitrification rates, N_2 may remain above saturation throughout diel sampling periods (McCutchan et al 2003; Pribyl et al. 2005). Where longer-term N_2 accumulation is likely (e.g., thermally stable systems with low gas exchange), the symbols at the top of Figs. 2 and 3 may be used to estimate detection limits. These symbols identify sce-

narios under which N_2 accumulation was not measurable. That is, measured concentrations did not exceed saturation concentrations by more than $1 \mu\text{mol N}_2 \text{ L}^{-1}$ (Fig. 2) or $4 \mu\text{mol N}_2 \text{ L}^{-1}$ (Fig. 3) after a period of 12 d. In the vast majority of cases, steady-state conditions were established within this time period. In some cases (largely in systems where the method is highly sensitive), N_2 accumulation may continue beyond 12 d. However, we use 12 d as an upper limit because longer term thermal stability and stability in discharge of shallow ($< 1 \text{ m}$ depth) streams seem unlikely. As well, the error associated with measuring factors such as gas transfer over this time period could be considerable. Interpretations based on 40 h accumulation or 12 d accumulation yield broadly similar trends—the method is not suited to measurement of low denitrification rates in shallow systems with moderate-high flow. In deeper systems, the method is more sensitive, but sensitivity declines as stream velocity, and associated turbulence, increases. Comparison of the two error terms indicates that very good analytical accuracy is critical to the ability to measure low, or even moderate, rates of denitrification.

The model-based analyses indicate that shallow streams (0.2 m) may represent systems in which low denitrification rates may be measured, but only at extremely low flow rates and with good analytical accuracy. Gas exchange rates at stream velocities of 0.02 m s^{-1} are predicted to be low enough that N_2 produced at low rates can be detected in the shallow water column relatively quickly, and denitrification rates $\leq 100 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ may be measurable (Fig. 2). However, careful sampling design may be required to account for poor mixing within such streams. At higher stream velocities, predictions of the wind and benthic reaeration models diverge quite markedly, and minimum measurable denitrification rates are increased. Rates of denitrification below $1000 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ should be measurable in shallow (0.2 m) streams with moderate flows ($0.16\text{--}0.32 \text{ m s}^{-1}$; Fig. 2) if analytical accuracy is high (within $1 \mu\text{mol N}_2 \text{ L}^{-1}$). Clearly, the method is not suited to measuring low denitrification rates in very shallow streams with very high velocity (0.64 m s^{-1}) where turbulence-induced gas exchange leads to rapid re-equilibration of water

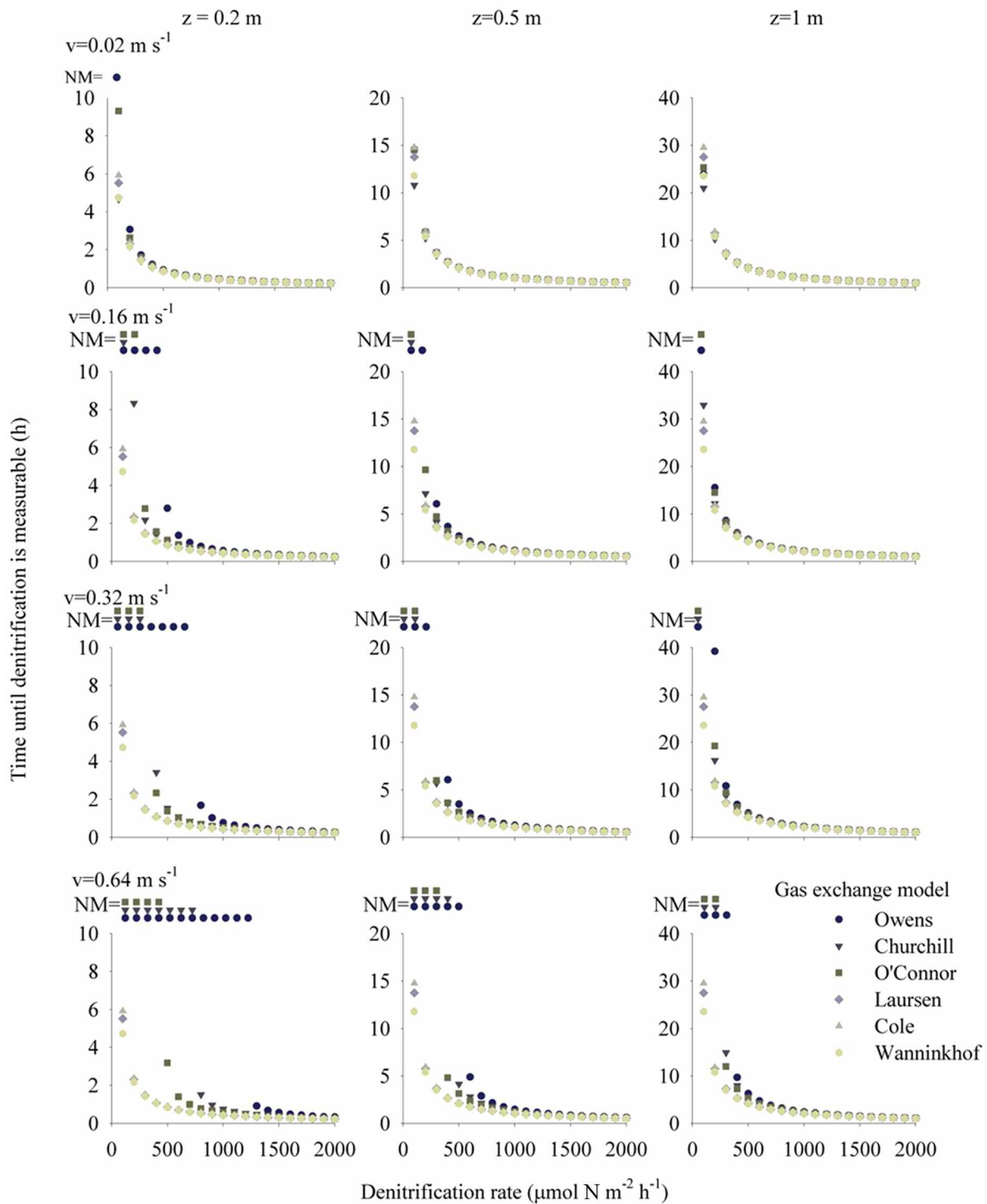


Fig. 2. Time required from model start (at saturation conditions) before denitrification is measurable under a suite of stream depths (columns) and stream velocities (rows), assuming analytical uncertainty of $1 \mu\text{M N}_2$. Base conditions of stable wind speed (2 m s^{-1}), temperature (20°C) and pressure (1008.3 hPa) were used. NM indicates that denitrification rates were not measurable after 12 d.

masses with the atmosphere. Within streams with depths of 0.5-1 m, denitrification rates $\leq 500 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ should be measurable even at the highest stream velocity tested (0.64 m s^{-1}), if analytical accuracy is high. In deeper systems with low

denitrification rates, the time until denitrification becomes measurable can be rather long (Figs. 2, 3), although such systems are likely to have greater thermal stability than shallow streams (Caissie 2006). If analytical accuracy is poor, this has

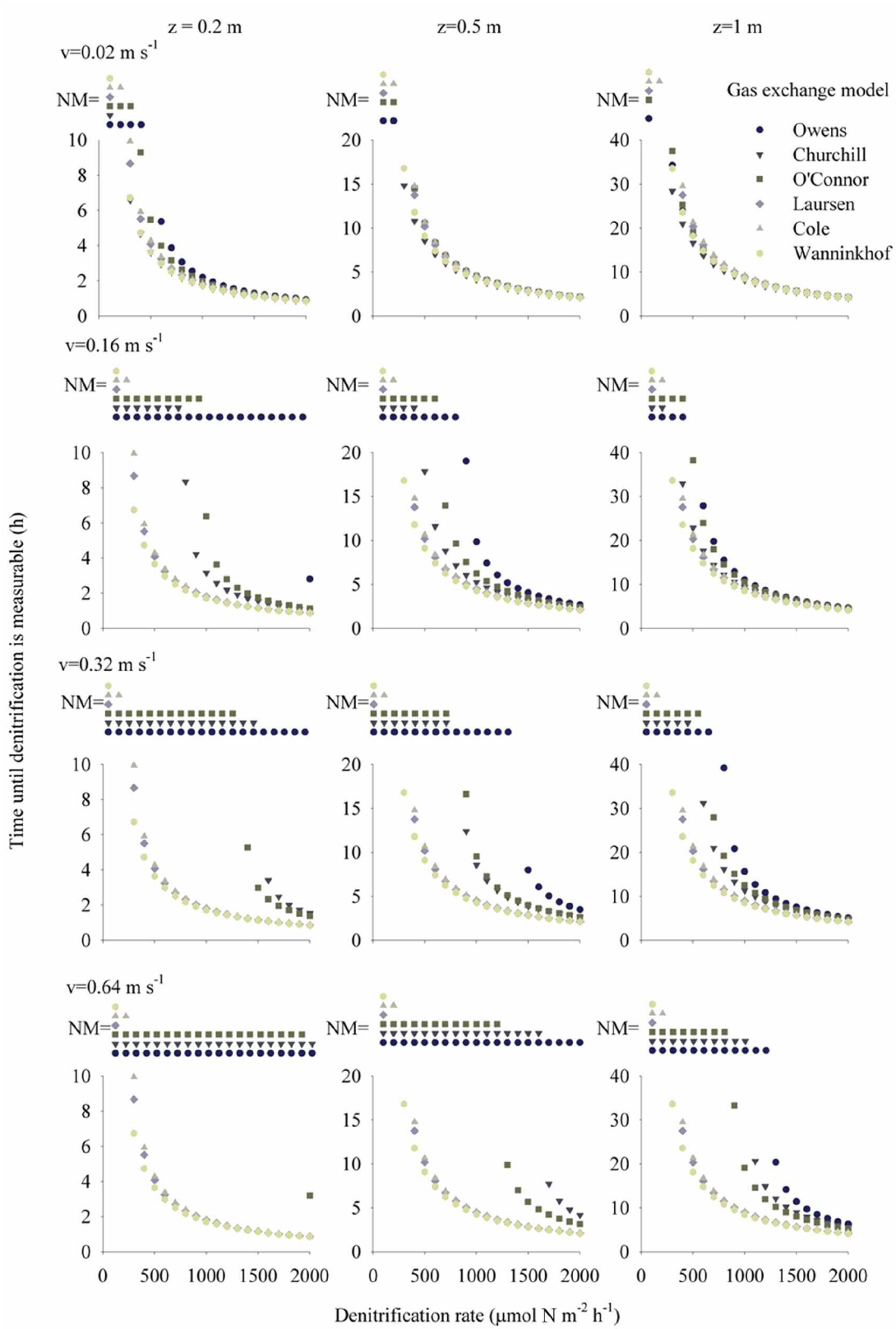


Fig. 3. Time required from model start (at saturation conditions) before denitrification is measurable under a suite of stream depths (columns) and stream velocities (rows), assuming analytical uncertainty of $4 \mu\text{M N}_2$. Base conditions of stable wind speed (2 m s^{-1}), temperature (20°C) and pressure (1008.3 hPa) were used. NM indicates that denitrification rates were not measurable after 12 d.

considerable implications for application of the method, particularly in systems with moderate to high stream velocity and in streams < 1 m in depth (Figs. 2 and 3).

The selection of benthic turbulence versus wind-based models (*sensu* Laursen and Seitzinger 2005) to estimate method sensitivity in candidate study streams is clearly important. The selected gas exchange models were developed for different environmental conditions, and researchers may wish to review these conditions (Web Appendix 2). Alternative models of benthic turbulence-induced gas exchange that incorporate factors such as surface roughness and slope may help further guide researchers in site selection. Likewise, models incorporating both benthic and wind-based gas exchange may be suitable in some systems (Chu and Jirka 2003). However, no model-based approach is adequate for in-field application of the open-channel method, except in very deep systems where wind-based models may be sufficiently accurate (Yan et al. 2004) and direct measurements are logistically difficult. Although the model analysis of Laursen and Seitzinger (2005) in conjunction with our findings can guide researchers in site selection, we suggest that the next step in ensuring appropriate site selection is detailed site surveys to assess spatial variability, and in particular, to reveal the presence of high gas exchange reaches, such as reaches with high slopes or riffles, which may limit method sensitivity.

Finally, we note that many researchers will lack a priori knowledge of denitrification rates to allow them to make approximations of whether the method is likely to succeed in individual study systems. Published denitrification rates from different systems may be used as a preliminary guide (e.g., Mulholland et al. 2004; Laursen and Seitzinger 2005). Surveys of N₂:Ar ratios may also be used to determine whether the ratio is considerably above the saturation value. Alternatively, preliminary measurements using other techniques, or a conservative approach to site selection is sensible.

One station versus two station approaches—While the one-station and two-station approaches are mathematically equivalent, important differences in the assumptions of these methods as well as logistical concerns associated with their implementation may dictate which approach is more suitable. Importantly, the one-station method incorporates a time-for-space substitution, where temporal patterns of concentration and solubility at an (unsampled) upstream location are assumed to be the same as those measured in the same parcel of water as it passes through the downstream sampling station.

Determining where to add tracers when measuring gas transfer coefficient is less straightforward for the one-station approach than the two-station approach. Gas exchange is a first-order reaction (i.e., exponential loss); hence the length of stream that is integrated by a concentration measurement can be calculated. Researchers may choose to use a metric such as Eq. 2 as a starting point in selecting reach length. Assuming a stream is supersaturated, Eq. 2 represents the length of reach

over which 95% of upstream gases will be released and 5% will remain in solution.

$$\text{Distance}_{5\%} = \frac{3U}{K} \quad (2)$$

where U is stream velocity, K is the gas transfer coefficient, and $\text{Distance}_{5\%}$ is the distance at which 5% of upstream gases remain in solution (Beaulieu et al. 2008; Chapra and Di Toro 1991). However, reach lengths calculated using this method are often quite long and may be impractical for tracer addition experiments.

There are likely to be important differences in the accuracy of modeled saturation conditions between one-station and two-station approaches. Systems with high diel temperature variation are not ideal for application of any open-channel denitrification measurements (Laursen and Seitzinger 2005), although as in dissolved oxygen studies, with appropriate modeling and careful measurements, the method may still be used (Butcher and Covington 1995). Using the one-station approach, installation of temperature and pressure loggers is simple, but as mentioned, incorporates the assumption that an upstream site has similar conditions. Within two-station studies, even with relatively low diel temperature variation, difficulty in measuring the temperature of a parcel of water moving downstream may lead to error in the estimation of saturation concentrations. Ideally, real-time measurements of temperature as the parcel of water passes downstream should be obtained (e.g., Smith et al. 2008); however interpolation may also provide adequate results.

Although one station versus two station comparisons have yielded similar results for measurements of photosynthesis and respiration, these comparisons have not been made for the open-channel denitrification method and may yield new insights into confidence in the open-channel method.

Measurement of gas transfer coefficient—One of the most critical parameters in determination of denitrification rates using this method is the measurement of rates of air-water gas transfer. Modeling this parameter is not adequate for accurate determination of denitrification rates in most systems. Efforts should be made to ensure the measurement of gas transfer coefficient reflects the period of sampling and study reach as closely as possible (Web Appendix 1—Table 2). Whereas stability in the gas transfer coefficient over time has been shown in some streams and rivers (e.g., Pribyl et al. 2005), others show very high seasonal and spatial variability (Hope et al. 2001). Using the two-station approach, the gas transfer coefficient can be measured to exactly align with N₂ sampling in space and time. Using the one-station approach, the measurement should be designed to reflect rates of gas transfer for the stream length over which gas concentrations are integrated. The importance of diel changes in wind speed and potential for resulting bias in measured gas transfer rate should also be assessed.

The measurement of gas transfer coefficient using direct tracer additions is a widely used, relatively straightforward

technique within systems of low water mass (see Hibbs et al. 1998; Kilpatrick et al. 1989). Various other methods exist for estimating the gas transfer coefficient, each with their own inherent assumptions and errors. Methods include diel O_2 change (Odum 1956; Streeter and Phelps 1925; Venkiteswaran et al. 2007), controlled flux techniques (see Jähne and Haußecker 1998), or the use of Ar anomalies (Laursen and Seitzinger 2002, 2004), where gas transfer coefficient is estimated based on the deviation of Ar from saturation values. The Ar anomaly method has been used in open-channel denitrification measurements (Laursen and Seitzinger 2002), and incorporates many of the same assumptions. Whereas this appears to be an effective means of estimating gas transfer coefficient in some ecosystems, it may be less accurate than direct tracer measurements (Web Appendix 1—Table 2). Irrespective of the method selected to measure gas transfer coefficient, some estimate of the error associated with the measurement is required.

Diel variation—Significant diel changes in water temperature occur in most aquatic systems with low thermal masses, leading to changes in gas solubility, air-water gas transfer coefficient, and rates of biological processes. The importance of these diel temperature changes in open-channel denitrification measurements was noted by Laursen and Seitzinger (2005) who recommended selection of sites, or times, when thermal stability was maximal (Web Appendix 1—Table 2). If all parameters are adequately constrained, the physical factors associated with diel temperature changes will not significantly affect measurement of denitrification rates. However, where rates of temperature variation are high, more frequent sampling is required to account for changes in physical drivers of gas flux. As well, changes in biological rates driven by changes in temperature are more likely to occur. As a result, where temperature changes are significant, mathematical consideration of how temperature may affect denitrification rate is advised, rather than model-fitting assuming a constant denitrification rate (Butcher and Covington 1995; Web Appendix 1—Table 2).

The role of physical gas fluxes from sediments is also worthy of consideration. In systems with strong seasonal temperature variation, but relatively stable daily thermal conditions, physical N_2 fluxes between sediments and overlying water can be sufficiently high to lead to error in denitrification measurements (Lamontagne and Valiela 1995). In lower order systems, this effect could be exhibited to some extent over a diel period, depending on sediment permeability and stream heat budgets.

Monitoring wind speed is recommended (Web Appendix 1—Table 2) due to potential effects of diel variation on gas transfer coefficient. Diel variation in discharge is relatively common in rivers (Lundquist and Cayan 2002) and is often driven by variation in evapotranspiration and infiltration. While variation in discharge is likely to have some effect on reaeration rates, the more important implication is that groundwater dynamics may be temporally variable during open-channel measurements, complicating N_2 mass balances.

Groundwater—Groundwater inputs, if not adequately characterized, could lead to significant error in denitrification measurements (see Laursen and Seitzinger 2005) because groundwater is frequently supersaturated with N_2 , and because temperature often differs between ground and surface waters. Where groundwater or tile drain inputs are known to occur along a stream, Eq. 2 (or an alternate first order loss calculation with a different endpoint such as 1% or 10%) can be used to help constrain the region in which gas concentrations are affected by the inflow. Sites that lack significant groundwater N_2 inputs are best for application of this method. Application of the method to sites with groundwater inputs relies upon adequate spatial and temporal characterization of the inputs and ensuring the inputs are well mixed at sampling points (Web Appendix 1—Table 2). Groundwater outputs are less problematic, as they represent the loss of a mass of water with a N_2 concentration equivalent to that of the stream.

A final scenario is situations where groundwater is lost and gained along a reach. This could effectively disguise groundwater inputs if researchers adopt the commonly used incremental stream flow method, where upstream and downstream discharge are compared to identify net groundwater inputs or losses. In cases where groundwater dynamics are not well known, groundwater inflows along a reach can be made concurrently with measurements of gas transfer coefficient by measuring dilution of the conservative tracer or using alternative approaches (Kalbus et al. 2006). Direct measurement of gross groundwater inflow would significantly reduce uncertainty in the application of this method. Alternatively, in-stream piezometers could be used to indicate the direction of groundwater flow.

A final note regarding groundwater inputs relates to the mixing of two water masses with different temperatures. Because saturation conditions vary nonlinearly, if two water masses at equilibrium mix, the resulting water mass will have a N_2 concentration that deviates at least slightly from saturation concentrations. For example, if 10% of stream flow is derived from N_2 -saturated groundwater at 10°C, but the stream is at saturation at 20°C, instantaneous mixing of these water masses would result in a temperature of 19°C, but a N_2 concentration of 540.1 μM . This is 1.9 μM above saturation for that water temperature and could be misinterpreted as a denitrification signal. Although a correction for the excess N_2 in groundwater is relatively straightforward, determining how to handle mixing of water masses with different temperatures is more difficult and will depend on how quickly the mixed water mass is subsequently heated or cooled. If the excess N_2 source can be identified and corrected for, the spatial effect on temperature measurements can also be constrained and may be present only over a limited area.

Mixing—Both the one-station and two-station approaches assume that the water column is well mixed. This may be a difficult assumption to fulfill in broad, slow moving rivers, where lateral variation in gas concentrations or temperature

may occur, or in systems with significant groundwater inflows or macrophytes. Problems of incomplete mixing may be addressed by assessing this heterogeneity in sampling design; however, uncertainty in such studies would increase, as the volume, N_2 concentration, and temperature of water masses across stream transects would have to be characterized. The mixing of water masses with different temperatures also has implications for estimation of N_2 saturation.

Ebullition and N_2 fixation as loss mechanisms for N_2 —Nitrogen fixation is a sink for dissolved N_2 , hence estimates of denitrification using the open-channel method are often referred to as net denitrification (i.e., gross denitrification minus N_2 fixation). Within fluvial systems, the importance of N_2 fixation to N budgets is very much an open question. Very few measurements of this process have been made using any method, and even fewer measurements have been made concurrently with measurements of other N uptake processes (Marcarelli et al. 2008). The difference between net and gross denitrification will depend on the study system, and approximations of the differences among systems cannot be made without further study. Nonetheless, net denitrification is an important measurement for studies focused on constraining N budgets, although understanding environmental controls on this composite measurement is more difficult. In contrast to N_2 fixation, anammox can lead to the production of N_2 , although the importance of this process in fluvial systems is not known.

The open-channel method, as described here does not incorporate bubble- or plant-mediated N_2 fluxes. Bubble-mediated gas fluxes are poorly constrained in lotic systems, but a recent study in the South Platte River showed that ebullition may transport up to $0.44 \text{ g N m}^{-2} \text{ d}^{-1}$ from sediments to the atmosphere (Higgins et al. 2008). By comparing these estimates to past measurements of diffusive flux in the system, Higgins et al. (2008) estimate 9% to 16% of N_2 flux may be driven by bubble-mediated transport. The importance of bubbles as an error term will depend on rates of bubble release, bubble N_2 concentration, and whether bubble N_2 was of atmospheric origin, or produced via denitrification. Systems with high bubble fluxes are best avoided for the application of this method. The importance of macrophytes as conduits of N_2 merits further investigation.

Measurement of N_2 concentrations—Membrane-inlet mass spectrometry allows high precision measurement of dissolved N_2 concentrations via either direct measurement of the N_2 signal or the use of N_2 to argon (N_2 :Ar ratios). Ratio measurements have greater precision (coefficient of variation of $< 0.05\%$; Kana et al. 1994) than measurement of the N_2 signal (coefficient of variation $< 0.5\%$; Kana et al. 1994), and are well-suited to measurement of denitrification in chambers, where Ar concentrations can generally be assumed to equal saturation concentrations. Based on knowledge of Ar, N_2 concentrations can then be calculated. In either case (measuring N_2 directly, or N_2 :Ar), obtaining three to four replicate samples is typically suf-

ficient to obtain good precision (Web Appendix 1—Table 2). Within open systems, particularly where groundwater inputs are significant or where diel temperature changes occur, Ar concentrations frequently deviate from saturation, necessitating their direct measurement (Laursen and Seitzinger 2002).

Calibration of MIMS is typically performed using one or more air-equilibrated standards at known temperature, pressure, and salinity. Method accuracy is typically good (Kana et al. 1994), but very high accuracy is required for application of the open reach method (Figs. 2 and 3). The majority of MIMS-based denitrification measurements are from closed chambers, so slight errors in instrument calibration will have lesser consequences than in the open-system method. Multiple standards should be used to ensure adequate calibration when applying the open-channel method, and analytical uncertainty terms for uncertainty analysis should be chosen carefully (Web Appendix 1—Table 3). Sample handling is an additional concern, as changes in concentration during storage may occur (McCutchan et al. 2003). Ideally, samples should be obtained in vials with ground glass stoppers and stored underwater at 1 to 2°C below ambient stream temperatures. Finally, N_2 may be scavenged by O_2 in some mass spectrometers, resulting in NO^+ formation in the ion source, and a decrease in the measured N_2 signal (Eyre et al. 2002). Researchers should test for this effect and determine whether the installation of a heated copper reduction column is necessary to remove O_2 (Eyre et al. 2002; Kana and Weiss 2004).

Time scale of sampling—Time step can be of critical importance both in modeling and ensuring the sampling program is adequately capturing in situ N_2 dynamics. Data loggers can be employed to characterize saturation conditions at very short time intervals; however, N_2 sampling is performed at longer time intervals due to the effort involved in field sampling and lab analyses. In one-station or diel approaches, the ability of the model to reproduce the tops and the bottoms of concentration curves is a good test of model accuracy. Samples obtained near daily maximum and minimum temperatures are therefore particularly valuable in assessing model fit.

Assumption of constant denitrification rate—The model is applied to generate an average denitrification rate that results in the best fit between measured and predicted N_2 concentrations over the study period. That is, the method assumes a single constant time and space-integrated denitrification rate. Because denitrification rates can vary with numerous factors including temperature, oxygen status, and substrate type (Risgaard-Petersen et al. 1994; Kemp and Dodds 2002a,b), this assumption may not hold true in systems with significant temporal or spatial variability in these factors. For example, diel variation in denitrification rates may occur (An and Joye 2001). In four systems where the open-channel method has been applied, short-term variation in denitrification rates was significant (Laursen and Seitzinger 2004; Harrison et al. 2005), but it was not significant in a fifth (McCutchan et al. 2003).

Modeling error—The open-channel method calculates denitrification based on the following measured input terms: morphometry (depth, width), stream velocity, time, measured concentrations, equilibrium concentrations, measured temperature, and gas transfer coefficient (Laursen and Seitzinger 2002). Equilibrium concentrations are determined from solubility equations using measured temperature and salinity. Equilibrium concentrations should also be corrected for atmospheric pressure. Method uncertainty is a function of uncertainty in the measured variables and the parameters derived from these measurements. Method uncertainty is also affected by assumptions of the method, including the use of a single (temporally and spatially integrated) denitrification rate.

Many researchers have wisely attempted to constrain the error in reported denitrification rates. However, the handling of error terms has varied among research groups. In several studies, error was assumed to be additive, and analytical error, errors in gas transfer measurements, Schmidt number exponent, and morphometry have been incorporated into estimates (Laursen and Seitzinger 2002; Smith et al. 2008). In a deeper river, error in gas transfer and analytical uncertainty were considered (Yan et al. 2004). In other cases, Monte Carlo analyses have been used incorporating analytical error, calculated solubility (due to temperature and pressure measurements), channel depth, and groundwater inputs (McCutchan et al. 2003). Where error within the method was not explored mathematically, comparisons among methods or times have been used instead (Pribyl et al. 2005).

Monte Carlo-based analyses are likely to provide the most robust estimate of error (Beck 1987). In Monte Carlo uncertainty analysis, the first step is the identification of uncertain model parameters. In Web Appendix 1 (Table 3), we present a suite of error terms for the method, and note which model

input parameters will be affected by these measurements. The next step in Monte Carlo uncertainty analysis is assigning ranges and distributions for parameters. Where a single value is most likely to be true—for example, in the measurement of temperature—the measured value may be used to define the mean of a distribution, and specifications reported by the manufacturer can be used to place bounds on the distribution. If a parameter is highly insensitive (e.g., salinity in a freshwater river), it may be set as fixed; however the findings of the uncertainty analysis will then depend upon the assumption of absolute certainty in that parameter. Assigning parameter uncertainty ranges and distributions is subjective, but the implications of different decisions can be easily explored by rerunning the analysis to reflect different assumptions. If parameters covary, covariances structures should be accounted for, or inflated estimates of uncertainty may result (Beck 1987; Skeffington 2006).

Once the parameter distributions are assigned, software packages can then be used to run the model iteratively, repeatedly taking random (or stratified random) samples from the assigned parameter distributions. The model may be run hundreds or thousands of times to generate a range of denitrification rates, which can be used to estimate the uncertainty in open-channel measurements.

Alternative approaches to measuring denitrification—The open-channel method is a powerful means of measuring denitrification in some systems, but its use is technically challenging and not suited to all ecosystems or research questions (Fig. 4, Table 1). Reach scale denitrification estimates via open-channel measurements, whole-stream ^{15}N addition experiments, natural abundance nitrate isotopes or classical mass balances are extremely useful in obtaining denitrification rate measurements at scales relevant to ecosystem models. However, each is limited in its applica-

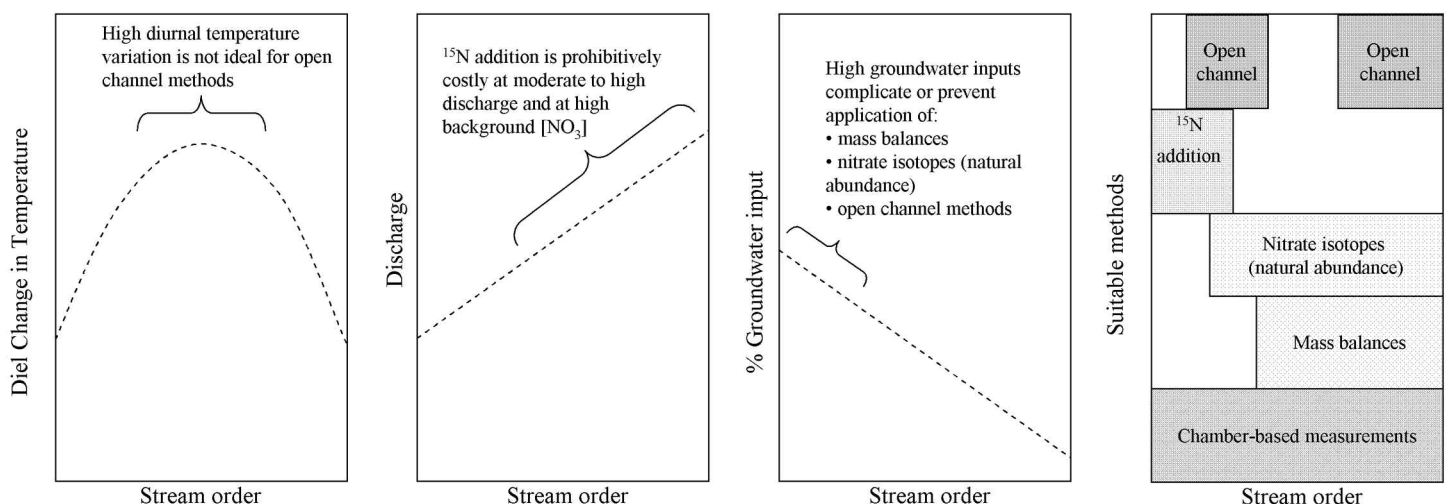


Fig. 4. Generalized suitability of different denitrification methods based on stream order (increasing from left to right). The diel temperature curve is based on Caissie (2006). Chamber-based methods refer to in-stream or in-lab techniques.

tion: the open-channel method largely by detection limits and groundwater inflow, the ^{15}N addition method by high cost of isotopes in high discharge systems or systems with high background nitrate, natural abundance isotopes by the need to characterize all nitrate sources and ensure all fractionating processes are adequately modeled, and mass balances by the need to measure all N fluxes other than denitrification, as well as changes in storage (Fig. 4, Table 1). In contrast, chamber-based methods tend to be more flexible and easier to apply across a range of ecosystem types than mass balance, natural abundance isotope, or open-channel approaches. Chamber-based methods allow assessment of heterogeneity at a fine spatial scale. However, scaling chamber-based estimates to reach and ecosystem scales can be error prone, and chambers can lead to the creation of unnatural conditions or allow shifts in microbial communities that may bias results (Table 1).

Methodological limitations have slowed progress in our understanding of denitrification (Groffman et al. 2006; Davidson and Seitzinger 2006). While we agree with the assessment of Groffman and others that the open-channel method is a valuable tool to help constrain denitrification rates, and it is likely to be widely applied due to low costs and increasing availability of analytical techniques, it is clear that successful application requires careful site selection, highly accurate N_2 measurements, attention to sensitive parameters such as measurement of gas exchange rate, and modeling that comprehensively addresses methodological uncertainty (Web Appendix 1—Table 3).

Recommendations

The open-channel method has contributed to improved understanding of denitrification as a N removal process at environmentally relevant spatial scales. Broader application of the method is feasible as analytical equipment becomes more widely available. However, successful application of the method is limited by our ability to reliably distinguish physical and biotic N fluxes. As a result, the uncertainty associated with measurements can be large. We recommend a conservative approach to site selection, and emphasize that the method may not be suitable for shallow streams or fast flowing systems, unless denitrification rates are high. We concur with Laursen and Seitzinger (2005) that deep rivers and periods of high wind may also be unsuitable for application of this method. However, measurements in deep rivers may be feasible via modifications to the method that rely upon longer-term gas accumulation and estimates of gas transfer (sensu Yan et al. 2004). All methods of measuring denitrification have inherent bias, either in the types of systems in which they may be applied (Fig. 4) or in potential artefacts associated with the methods themselves (e.g., acetylene block). For this reason, comparisons among methods should be applied, when possible, to foster a better understanding of the limitations of each method.

References

- An, S., and S. B. Joye. 2001. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnol. Oceanogr.* 46:62-74.
- Beaulieu, J. J., C. P. Arango, S. K. Hamilton, and J. L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern United States. *Glob. Change Biol.* 14:878-94 [doi:10.1111/j.1365-2486.2007.01485.x].
- Beck, M. B. 1987. Water quality modeling: a review of the analysis of uncertainty. *Water Resour. Res.* 23:1393-1441 [doi:10.1029/WR023i008p01393].
- Butcher, J. B., and S. Covington. 1995. Dissolved-oxygen analysis with temperature dependence. *J. Env. Eng.* 121:756-759 [doi:10.1061/(ASCE)0733-9372(1995)121:10(756)].
- Caissie, D. 2006. The thermal regime of rivers: a review. *Freshwat. Biol.* 51:1389-1406. [doi:10.1111/j.1365-2427.2006.01597.x].
- Chapra, S. C., and D. M. Di Toro. 1991. Delta method for estimating primary production, respiration, and reaeration in streams. *J. Env. Eng.* 117:640-655 [doi:10.1061/(ASCE)0733-9372(1991)117:5(640)].
- Chu, C. R., and G. H. Jirka. 2003. Wind and stream flow induced reaeration. *J. Env. Eng.* 129:1129-1136 [doi:10.1061/(ASCE)0733-9372(2003)129:12(1129)].
- Churchill, M. A., H. L. Elmore, and R. A. Buchingham. 1962. The prediction of stream reaeration rates. *J. Sanit. Eng. Div.* 88:1-46.
- Cole, J. J., and N. F. Caraco. 1998. Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF_6 . *Limnol. Oceanogr.* 43:647-656.
- Cox, B. A. 2003. A review of dissolved oxygen modelling techniques for lowland rivers. *Sci. Tot. Environ.* 314:303-334 [doi:10.1016/S0048-9697(03)00062-7].
- Davidson, E. A., and S. Seitzinger. 2006. The enigma of progress in denitrification research. *Ecol. Appl.* 16:2057-2063 [doi:10.1890/1051-0761(2006)016[2057:TEOPID]2.0.CO;2].
- Eyre, B. D., S. Rysgaard, T. Dalsgaard, and P. B. Christensen. 2002. Comparison of isotope pairing and N_2 :Ar methods for measuring sediment denitrification – assumptions, modifications, and implications. *Estuaries* 25:1077-1087 [doi:10.1007/BF02692205].
- Groffman, P. M., and others. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. *Ecol. Appl.* 16:2091-2122 [doi:10.1890/1051-0761(2006)016[2091:MFMDDA]2.0.CO;2].
- Gualtieri, C., P. Gualtieri, and G. P. Doria. 2002. Dimensional analysis of reaeration rate in streams. *J. Environ. Eng.* 128:12-18 [doi:10.1061/(ASCE)0733-9372(2002)128:1(12)].
- Hamme, R. C., and S. R. Emerson. 2004. The solubility of neon, nitrogen and argon in distilled water and seawater. *Deep-Sea Res.* 51:1517-1528.
- Harrison, J. A., P. A. Matson, and S. E. Fendorf. 2005. Effects of

- a diel oxygen cycle on nitrogen transformations and greenhouse gas emissions in a eutrophied subtropical stream. *Aquat. Sci.* 67:308-315 [doi:10.1007/s00027-005-0776-3].
- Hibbs, D. E., K. L. Parkhill, and J. S. Gulliver. 1998. Sulfur hexafluoride gas tracer studies in streams. *J. Env. Eng.* 124:752-760 [doi:10.1061/(ASCE)0733-9372(1998)124:8 (752)].
- Higgins, T. M., J. H. McCutchan Jr., and W. M. Lewis Jr. 2008. Nitrogen ebullition in a Colorado plains river. *Biogeochemistry* 89:367-377 [doi:10.1007/s10533-008-9225-4].
- Ho, D. T., L. F. Bliven, R. Wanninkhof and P. Schlosser. 1997. The effect of rain on air-water gas exchange. *Tellus Ser. B.* 49:149-158 [doi:10.1034/j.1600-0889.49.issue2.3.x].
- Hope, D., S. M. Palmer, M. F. Billett, and J. J. C. Dawson. 2001. Carbon dioxide and methane evasion from a temperate peatland stream. *Limnol. Oceanogr.* 46:847-857.
- Jähne, B. 1987. On the parameters influencing air-water gas exchange. *J. Geophys. Res.* 92:1937-1950 [doi:10.1029/JC092iC02p01937].
- Jähne, B., and H. Haußecker. 1998. Air-water gas exchange. *Annu. Rev. Fluid Mech.* 30:443-468 [doi:10.1146/annurev.fluid.30.1.443].
- Jha, R., C. S. P. Ojha, and K. K. S. Bhatia. 2001. Refinement of predictive reaeration equations for a typical Indian river. *Hydrol. Proc.* 15:1047-1060 [doi:10.1002/hyp.177].
- Kalbus, E., F. Reinstorf, and M. Schirmer. 2006. Measuring methods for groundwater, surface water and their interactions: a review. *Hydrol. Earth. Syst. Sci.* 10:873-887 [doi:10.5194/hess-10-873-2006].
- Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennett, and J. C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N₂, O₂, and Ar in environmental water samples. *Anal. Chem.* 66:4166-4170 [doi:10.1021/ac00095a009].
- , and D. L. Weiss. 2004. Comment on "Comparison of isotope pairing and N₂ : Ar methods for measuring sediment denitrification." *Estuaries Coasts* 27:173-176 [doi:10.1007/BF02803571].
- , J. C. Cornwell, and L. Zhong. 2006. Determination of denitrification in the Chesapeake Bay from measurements of N₂ accumulation in bottom water. *Estuaries Coasts* 29:222-231 [doi:10.1007/BF02781991].
- Kellman, L. 2004. Nitrate removal in a first-order stream: reconciling laboratory and field measurements. *Biogeochemistry* 71:89-105 [doi:10.1007/s10533-004-4318-1].
- , and C. Hillaire-Marcel. 1998. Nitrate cycling in streams: Using natural abundances of NO₃⁻-δ¹⁵N to measure *in-situ* denitrification. *Biogeochemistry* 43:273-292 [doi:10.1023/A:1006036706522].
- Kemp, M. J., and W. K. Dodds. 2002a. Comparisons of nitrification and denitrification in prairie and agriculturally influenced streams. *Ecol. Appl.* 12:998-1009 [doi:10.1890/1051-0761(2002)012[0998:CONADI]2.0.CO;2].
- , and W. K. Dodds. 2002b. The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata. *Limnol. Oceanogr.* 47:1380-1393.
- Kilpatrick, F. A., R. E. Rathbun, N. Yotsukura, G. W. Parker, and L. L. DeLong. 1989. Chapter A18: Determination of stream reaeration coefficients by use of tracers. U.S. Geological Survey. (Techniques of water-resources investigations of the United States Geological Survey).
- Lamontagne, M. G., and I. Valiela. 1995. Denitrification measured by a direct N₂ flux method in sediments of Waquoit Bay, MA. *Biogeochemistry* 31:63-83 [doi:10.1007/BF00000939].
- Laursen, A., and S. Seitzinger. 2005. Limitations to measuring riverine denitrification at the whole reach scale: effects of channel geometry, wind velocity, sampling interval, and temperature inputs of N₂-enriched groundwater. *Hydrobiol.* 545:225-236 [doi:10.1007/s10750-005-2743-3].
- Laursen, A. E., and S. P. Seitzinger. 2002. Measurement of denitrification in rivers: an integrated, whole reach approach. *Hydrobiologia* 485:67-81 [doi:10.1023/A:1021398431995].
- , and ———. 2004. Diurnal patterns of denitrification, oxygen consumption and nitrous oxide production in rivers measured at the whole-reach scale. *Freshwat. Biol.* 49:1488-1458 [doi:10.1111/j.1365-2427.2004.01280.x].
- Lundquist, J. D., and D. R. Cayan. 2002. Seasonal and spatial patterns in diurnal cycles in streamflow in the western United States. *J. Hydrometeor.* 3:591-603 [doi:10.1175/1525-7541(2002)003<0591:SASPID>2.0.CO;2].
- Marcarelli, A. M., M. A. Baker, and W. A. Wurtsbaugh. 2008. Is in-stream N₂ fixation an important N source for benthic communities and stream ecosystems? *J. N. Am. Benthol. Soc.* 27:186-211 [doi:10.1899/07-027.1].
- Mackay, D., and A. T. K. Yeun. 1983. Mass-transfer coefficient correlations for volatilization of organic solutes from water. *Environ. Sci. Tech.* 17:211-217 [doi:10.1021/es00110a006].
- McCutchan, J. H., J. F. Saunders, A. L. Pribyl, and W. M. Lewis. 2003. Open-channel estimation of denitrification. *Limnol. Oceanogr. Methods* 1:74-81.
- Melching, C. S., and H. E. Flores. 1999. Reaeration equations derived from US geological survey database. *J. Environ. Eng.* 125:407-414 [doi:10.1061/(ASCE)0733-9372(1999)125:5(407)].
- Moog, D. B., and G. H. Jirka. 1998. Analysis of reaeration equations using mean multiplicative error. *J. Env. Eng.* 124:104-110 [doi:10.1061/(ASCE)0733-9372(1998)124:2(104)].
- Mulholland, P. J., H. M. Valett, J. R. Webster, S. A. Thomas, L. W. Cooper, and S. K. Hamilton. 2004. Stream denitrification and total nitrate uptake rates measured using a field ¹⁵N tracer addition approach. *Limnol. Oceanogr.* 49:809-820.
- Nielsen, L. P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microb. Ecol.* 86:357-362 [doi:10.1111/j.1574-6968.1992.tb04828.x].
- O'Connor, D. J., and W. E. Dobbins. 1958. Mechanism of reaeration in natural streams. *Amer. Soc. Civil Eng. Trans.* 123:641-684.

- Odum, H. T. 1956. Primary production in flowing waters. *Limnol. Oceanogr.* 1:102-117 [doi:10.4319/lo.1956.1.2.0102].
- Owens, M., R. W. Edwards, and J. W. Gibbs. 1964. Some reaeration studies in streams. *Int. J. Air Water Poll.* 8:469-486.
- Pribyl, A. L., J. H. McCutchan, W. M. Lewis, and J. F. Saunders. 2005. Whole-system estimation of denitrification in a plains river: a comparison of two methods. *Biogeochemistry* 73:439-455 [doi:10.1007/s10533-004-0565-4].
- Raymond, P. A., and J. J. Cole. 2001. Gas exchange in rivers and estuaries: Choosing a gas transfer velocity. *Estuaries* 24:312-317 [doi:10.2307/1352954].
- Risgaard-Petersen, N., S. Rysgaard, L. P. Nielsen, and N. P. Revsbech. 1994. Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnol. Oceanogr.* 39:573-579.
- Schwarzenbach, R. P., P. M. Gschwend, and D. M. Imboden. 1993. *Environmental organic chemistry*. Wiley. See p. 235.
- Sjodin, A. L., W. M. Lewis Jr., and J. F. Saunders III. 1997. Denitrification as a component of the nitrogen budget for a large plains river. *Biogeochemistry* 39:327-342 [doi:10.1023/A:1005884117467].
- Skeffington, R. A. 2006. Quantifying uncertainty in critical loads: (A) literature review. *Water Air Soil Pollut.* 169:3-24 [doi:10.1007/s11270-006-0382-6].
- Smith, T. E., A. E. Laursen, and J. R. Deacon. 2008. Nitrogen attenuation in the Connecticut River, northeastern USA; a comparison of mass balance and N₂ production modeling approaches. *Biogeochemistry* 87:311-323 [doi:10.1007/s10533-008-9186-7].
- Streeter, H. W., and E. B. Phelps. 1925. A study of the pollution and natural purification of the Ohio River. III. Factors concerned in the phenomena of oxidation and reaeration. United States Public Health Service, U.S. Department of Health, Education, and Welfare. Public health bulletin no. 146, February 1925. Reprinted by USDHEW, PHA, 1958.
- Thamdrup, B., and T. Dalsgaard. 2002. Production of N₂ through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Appl. Env. Microbiol.* 68:1312-1318.
- Thomann, R. V., and J. A. Mueller. 1987. *Principles of surface water quality modeling and control: quality modeling and control*. Harper & Row.
- Venkiteswaran, J. J., L. I. Wassenaar, and S. L. Schiff. 2007. Dynamics of dissolved oxygen isotopic ratios: a transient model to quantify primary production, community respiration and air-water exchange in aquatic ecosystems. *Oecologia* 153:385-398 [doi:10.1007/s00442-007-0744-9].
- Wanninkhof, R. 1992. Relationship between wind-speed and gas-exchange over the ocean. *J. Geophys. Res. Oceans* 97:7373-7382 [doi:10.1029/92JC00188].
- Yan, W., A. E. Laursen, F. Wang, P. Sun, and S. P. Seitzinger. 2004. Measurement of denitrification in the Changjiang River. *Environ. Chem.* 1:95-98 [doi:10.1071/EN04031].

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