CONCEPTS & SYNTHESIS
EMPHASIZING NEW IDEAS TO STIMULATE RESEARCH IN ECOLOGY

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Looking back to look ahead: a vision for soil denitrification research

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Abstract. Denitrification plays a critical role in regulating ecosystem nutrient availability and anthropogenic reactive nitrogen (N) production. Its importance has inspired an increasing number of studies, yet it remains the most poorly constrained term in terrestrial ecosystem N budgets. We censused the peer-reviewed soil denitrification literature (1975–2015) to identify opportunities for future studies to advance our understanding despite the inherent challenges in studying the process. We found that only one-third of studies reported estimates of both nitrous oxide (N_2O) and dinitrogen (N_2) production fluxes, often the dominant end products of denitrification, while the majority of studies reported only net N_2O fluxes or denitrification potential. Of the 236 studies that measured complete denitrification to N_2, 49% used the acetylene inhibition method, 84% were conducted in the laboratory, 81% were performed on surface soils (0–20 cm depth), 75% were located in North America and Europe, and 78% performed treatment manipulations, mostly of N, carbon, or water. To improve understanding of soil denitrification, we recommend broadening access to technologies for new methodologies to measure soil N_2 production rates, conducting more studies in the tropics and on subsoils, performing standardized experiments on unmanipulated soils, and using more precise terminology to refer to measured process rates (e.g., net N_2O flux or denitrification potential). To overcome the greater challenges in studying soil denitrification, we envision coordinated research efforts based on standard reporting of metadata for all soil denitrification studies, standard protocols for studies contributing to a Global Denitrification Research Network, and a global consortium of denitrification researchers to facilitate sharing ideas, resources, and to provide mentorship for researchers new to the field.

Key words: *census; denitrification; dinitrogen; nitrous oxide; soil; terrestrial.*

Introduction

Denitrification is widely recognized as an important biogeochemical process in terrestrial ecosystems that is inherently difficult to study. As one of few processes that return reactive nitrogen (N) forms to the unreactive form of dinitrogen (N_2), it plays a critical role in regulating ecosystem nutrient availability and mitigating excessive anthropogenic production of reactive N (Galloway et al. 2004). When it does not proceed to completion, denitrification can lead to gaseous emissions of nitric oxide (NO), a smog precursor, and nitrous oxide (N_2O), a potent greenhouse gas and contributor to stratospheric ozone depletion (Firestone and Davidson 1989). Measuring soil N_2 production from denitrification is difficult in terrestrial ecosystems because of the high atmospheric background N_2 concentration (Groffman et al. 2006). Furthermore, spatial and temporal variability in instantaneous point measurements of denitrification leads to high uncertainty when scaling up the measurements in space and time (McClain et al. 2003, Groffman et al. 2009). The importance of soil denitrification has motivated many studies, but it remains the most poorly constrained term in terrestrial N budgets due to these inherent challenges (Seitzinger et al. 2006). Here we take stock of past soil denitrification studies to identify opportunities for future studies to advance our understanding of denitrification despite these challenges.

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Since the turn of the 20th century, denitrification has been studied as a potentially important fate of fertilizer N (Voorhees 1902). Nitrogen mass balance estimates have suggested that denitrification rates are indeed much higher in intensive agroecosystems than in natural ecosystems and non-intensive agroecosystems (Seitzinger et al. 2006). However, denitrification fluxes estimated by N mass balance remain uncertain due to the difficulty in measuring biological N fixation, which is the dominant input of N in unmanaged ecosystems (Galloway et al. 2004). For example, in the tropics where ecosystem-level biological N fixation rates are suspected to be higher than measured (Cleveland et al. 1999, Houlton et al. 2008, Hedin et al. 2009), N mass balance model predictions of low to moderate denitrification rates may be underestimates (Seitzinger et al. 2006, Bouwman et al. 2013). An isotope mass balance model, which estimates N losses from denitrification based on $\delta^{15}N$ differences between the soil pool and atmospheric inputs, suggests that denitrification rates in some areas of the tropics could be as high as those in intensive agricultural regions (Fig. 1; Wang et al. 2017). However, this isotopic approach also has high uncertainty associated with error propagation. Therefore, empirical denitrification measurements are needed to validate both N and isotope mass balance model predictions. Given that soil denitrification rates were thought to be relatively low in natural ecosystems, past studies may have had an ecosystem or geographic bias that exacerbates uncertainty in denitrification estimates for these ecosystems relative to agroecosystems.

Challenges associated with measuring soil N_2 production rates have hindered efforts to constrain the contribution of denitrification to terrestrial ecosystem N budgets. Despite numerous studies concluding that the commonly used acetylene inhibition technique underestimates denitrification rates, this method remains popular. Other methodologies commonly used in soil systems, such as ^{15}N-nitrate (NO_3^-) tracer or helium flow through core incubations, do not exhibit the same measurement bias but appear relatively limited in their adoption. Additionally, these methods are all predominantly laboratory based. The removal of soil from the in situ environment has long been recognized to cause a change in soil conditions that could alter measured denitrification rates from those exhibited in the field (Voorhees 1902). In addition, a discrete soil depth increment must be sampled such that the contribution of subsoil to ecosystem-scale fluxes may not be taken into account. In response to a highly cited call for improved methodologies for measuring soil N_2 production (Groffman et al. 2006), the ^{15}N-N_2O pool dilution technique was developed to measure in situ gross N_2O fluxes in the field (Yang et al. 2011). This method may not capture all N_2 production, such as from complete denitrification occurring within anaerobic soil microsites (Well and Butterbach-Bahl, 2013, Yang et al. 2013, Wen et al. 2016). However, it is currently the only method that provides much-needed field measurements of gross N_2O emissions and uptake under undisturbed conditions (Yang et al. 2011, Yang and Silver 2016a, b, Wen et al. 2017). Most recently, natural abundance $^{15}N^{15}N$ soil profiles were used to estimate in situ field rates of soil N_2 production by biological processes (Yeung et al. 2019). Although the adoption of new methodologies may be slow, analyzing trends in the use of older methodologies could provide insight into how we can accelerate this process.

Even if soil N_2 production could be easily measured, an understanding of controls on denitrification rates and the relative contribution of denitrification end products would be necessary to accurately scale up measurements

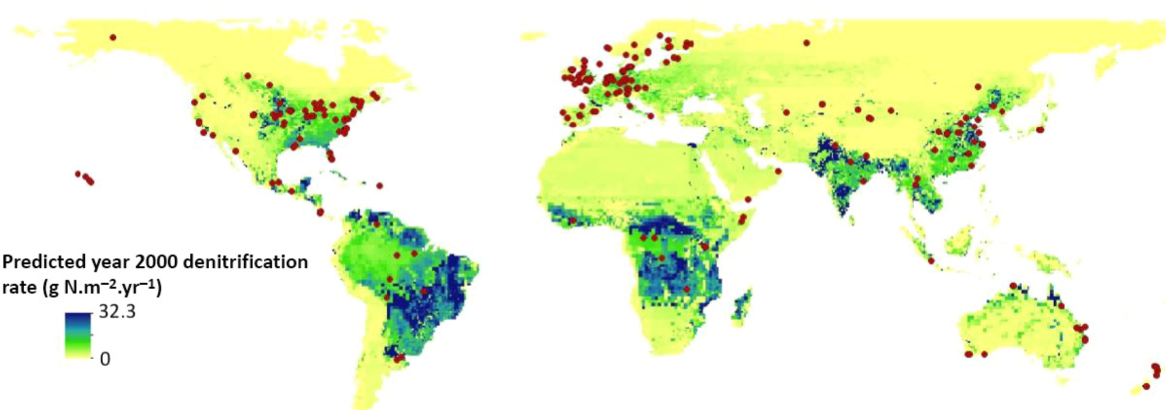

Fig. 1. Geographical distribution of study site locations for soil denitrification studies published during the period of 1975–2015 (red points) overlaid on the global distribution of denitrification rates predicted for the year 2000 by Wang et al. (2017) using an isotope mass balance model. This model estimates N outputs in 10 × 10 km grid cells by N mass balance and estimates the denitrification fraction of N outputs using the $\delta^{15}N$ soil N and atmospheric inputs (biological N fixation + atmospheric deposition) together with enrichment factors associated with leaching, denitrification gas loss, and ammonia volatilization.

for ecosystem N budgets. On the one hand, this understanding would inform when and where to conduct measurements to appropriately account for hot spots and hot moments of denitrification in empirically determined N budget estimates. On the other hand, this understanding could help improve the representation of denitrification in process models used to estimate denitrification rates at scales relevant to ecosystem N budgets (Boyer et al. 2006, Groffman et al. 2009). Treatment manipulations of soil moisture (or oxygen; O_2), NO_3^-, carbon (C), and pH have been used to demonstrate that these variables regulate denitrification rates and the relative contribution of denitrification end products (Firestone et al. 1979, Weier et al. 1993). While this approach is commonly used to determine the role of an independent variable in controlling a process of interest, the change in soil conditions caused by treatment manipulations represents a perturbation that can influence denitrification rates. Manipulative studies, therefore, can elucidate factors regulating the development of denitrification hot moments but may be less relevant for understanding spatial variability in denitrification rates likely driven by environmental heterogeneity. Relying on manipulative studies therefore could contribute to inaccuracy in ecosystem-level denitrification flux estimates.

How we have studied soil denitrification in the past has left gaps in knowledge that can be filled despite the inherent challenges in studying this process. To assess this, we performed a census of soil denitrification studies in the peer-reviewed literature over a 40-yr period (1975–2015) and used metadata from these studies to identify gaps in prior research efforts. Where research gaps align with major uncertainties in understanding soil denitrification, we make recommendations for how we can address those gaps. Since N_2O and N_2 are often major denitrification end products, we limited the census to studies that included empirical measurements of both N_2O and N_2 fluxes. Fluxes of NO, typically a minor end product of denitrification, are rarely measured along with N_2O and N_2 fluxes. Thus, we did not consider NO in this census. However, we acknowledge that NO production may represent a significant N source in some terrestrial ecosystems such as in chaparral forests and arid agricultural landscapes (Davidson and Kingerlee 1997, Almaraz et al. 2018).

Methods

We compiled a database of 236 upland soil denitrification studies published in peer-reviewed journals between 1975 and 2015 (Data S1: *Denitrification Studies*). We searched the following terms using Web of Science: "total denitrification," "nitrous oxide AND dinitrogen," "acetylene inhibition," "dinitrogen," "dinitrogen AND denitrification," "N_2O mole fraction," "N_2O yield," "nitrous oxide yield," and "denitrification AND soil." We excluded studies conducted in wetlands, engineered systems (e.g., bioreactors, pit toilets), and those that measured only potential denitrification rates or that reported net N_2O fluxes without estimates of N_2 production.

High variability in experimental parameters and the time scale of measurements precluded a quantitative meta-analysis of denitrification rates, thus we extracted only metadata from the studies in our database. Extracted metadata describing study site characteristics included the setting (i.e., laboratory or field), the type of ecosystem (i.e., desert, grassland, pasture, cropped agriculture, woodland, or shrubland/heath), the continent, and the Holdridge life zone based on mean annual temperature and precipitation for the study location (Holdridge 1967). Extracted metadata describing experimental designs included the method used to estimate N_2 production rates (Table 1); the variables that were manipulated, the type of denitrification rates reported (i.e., instantaneous vs. cumulative), the time frame of the study (hours–years), the soil depth increments sampled, and whether or not the soils were sieved. "Other" denitrification measurements include ^{15}N-N_2O pool dilution technique, isotopologues, membrane inlet mass spectrometer, or combined methods (e.g., N_2 flux determination by a ^{15}N-aided gas flow soil core system with an artificial atmosphere). We considered two types of manipulations: "treatment manipulations" describe manipulations imposed to test for treatment effects and "protocol-based manipulations" describe manipulations imposed on all samples as part of the denitrification measurement protocol. For example, when N was added to treatment samples and not to control samples to test for the effect of N addition, we classified the N addition as a treatment manipulation. In contrast, when N was added to all soil samples in the experiment as part of the ^{15}N tracer method, we classified the N addition as a protocol-based manipulation. Compost amendments were counted as C and N manipulations, whereas low-N organic matter (e.g., straw) amendments were counted as C manipulations only. Studies that included measurements at multiple locations, multiple experimental treatments, or that used multiple approaches were counted for each category that applied within a given parameter. Therefore, the total number of studies for a given parameter sometimes exceeds the 236 total number of studies in the database. We reported the data for each parameter as total counts per category or as the percentage contribution of each category to the total number of studies.

Results

The annual number of peer-reviewed studies published on upland terrestrial denitrification has grown over time (Appendix S1: Fig. S1). Acetylene inhibition was the most commonly used method for measuring N_2 production (57%), followed by the ^{15}N-NO_3^- tracer method (26%) and the direct measurement method (15%; Fig. 2a). Acetylene inhibition has consistently been the most commonly used method, accounting for 64%, 73%,

TABLE 1. Recommendations for standard metadata and units to be reported in all future soil denitrification studies.

Metadata	Units
Method used to measure N_2 production rates	see Table 2 for method names
Soil sample treatment	intact core, homogenized by gentle mixing, or homogenized by sieving
Control N_2 flux	$\mu g\ N \cdot g^{-1} \cdot d^{-1}$ or $mg\ N \cdot m^{-2} \cdot d^{-1}$
Control N_2O flux	$\mu g\ N \cdot g^{-1} \cdot d^{-1}$ or $mg\ N \cdot m^{-2} \cdot d^{-1}$
Soil NH_4^+ concentration	μg N/g
Soil NO_3^- concentration	μg N/g
Soil total N concentration	%
Soil organic C concentration	%
Antecedent soil moisture (gravimetric)	%
Experimental soil moisture (gravimetric)	%
Headspace oxygen	%
Soil temperature	°C
Soil pH in water (1:1 ratio of fresh soil mass to water volume)	logarithmic scale
Bulk soil density	g/cm^3
Soil texture	% sand/% silt/% clay
Topographic position	ridge, slope, or valley/depression

and 59% of studies in the 1980s, 1990s, and 2000s. The use of $^{15}N\text{-}NO_3^-$ tracers has decreased from 50% in the 1980s to 27% in the 2000s. In contrast, the use of direct measurements has increased from 7% in the 1990s, when these approaches were first introduced, to 19% in the 2000s. Most denitrification rates were reported as instantaneous rates (74%) rather than cumulative rates estimated over the entire study period (Fig. 2b).

Across all studies 78% performed treatment manipulations (Fig. 2c) and 71% performed protocol-based manipulations (Fig. 2d). Nitrogen (37%), water (22%), and C (21%) addition were the most common treatment manipulations (Fig. 2c) whereas N (32%), water (27%), and O_2 (22%) were the most common protocol-based manipulations (Fig. 2d). In 45% of studies, multiple treatment manipulations were imposed. Only 38% of studies used sieved soils (Fig. 2e). Of the 206 laboratory studies, few included soils sampled below 20 cm depth (19%), with 0–10 and 0–20 cm depths being the most common increments sampled (36% each; Fig. 2f).

We observed a geographical bias in the distribution of published studies. Most studies were conducted in North America and Europe (176 studies; 74%), with only three studies in South America and two studies in Africa (Fig. 2a). Managed ecosystems were studied more frequently than natural ecosystems, with cropped agricultural and pasture systems accounting for 39% and 14% of total studies, respectively (Fig. 3b). Unmanaged grasslands (15%) and woodlands (17%) were also commonly studied. Shrubland/heath (1%) and desert (0.4%) studies were rare (Fig. 3b). In terms of Holdridge life zones with respect to precipitation, one-half of the studies were in semiarid climates, whereas 32% of the studies were in subhumid climates (Fig. 3c). Only seven studies were conducted in wetter climates, ranging from humid to super-humid (Fig. 3c). With respect to temperature, 70% of studies were in temperate climates, while only 17% of studies were in subtropical and tropical climates (Fig. 3d). The majority of experiments were conducted in the laboratory (85%; Fig. 3e). The time frames of the studies were roughly equally distributed among time scales from hours to years (Fig. 3f).

DISCUSSION

Looking back

Our census revealed several gaps that must be addressed to advance our understanding of soil denitrification. Some of these gaps may not be surprising given the difficulty in measuring soil N_2 production rates, particularly in the field or in remote locations such as the tropics. However, there are other gaps, such as the lack of subsoil or unmanipulated soils data, that will be easier to address. Here we discuss these gaps as well as issues with standardization across studies that we identified in the process of conducting the census.

While the acetylene inhibition method has been known for decades to generate underestimates of denitrification rates (Knowles 1990, Groffman et al. 2006, Sgouridis et al. 2016), its continued prevalence suggests that its low cost and ease of use is a primary consideration in selecting a method for measuring soil N_2 production (Fig. 2b). In recent years, more studies have included direct measurements of N_2 production using a gas flow soil core incubation system, which is regarded as yielding more accurate estimates of denitrification rates than acetylene inhibition (Groffman et al. 2006). However, this method is being applied by only a handful of research groups worldwide who have the custom-built instrumentation necessary to use this technique (Data S1: *Denitrification Studies*). Similarly, the newer $^{15}N\text{-}N_2O$ pool dilution technique is employed by only a few research groups who possess the trace gas preconcentration units needed for N_2O analysis on isotope ratio mass spectrometers. The newest $^{15}N^{15}N$ technique for measuring in situ N_2 production rates requires expensive ultra-high-resolution mass spectrometers capable of clumped isotope analyses, also making this approach inaccessible to many researchers. The continued development of improved methodologies is exciting, but to truly advance soil denitrification research, the training and specialized instrumentation needed to use these methods must be more broadly accessible.

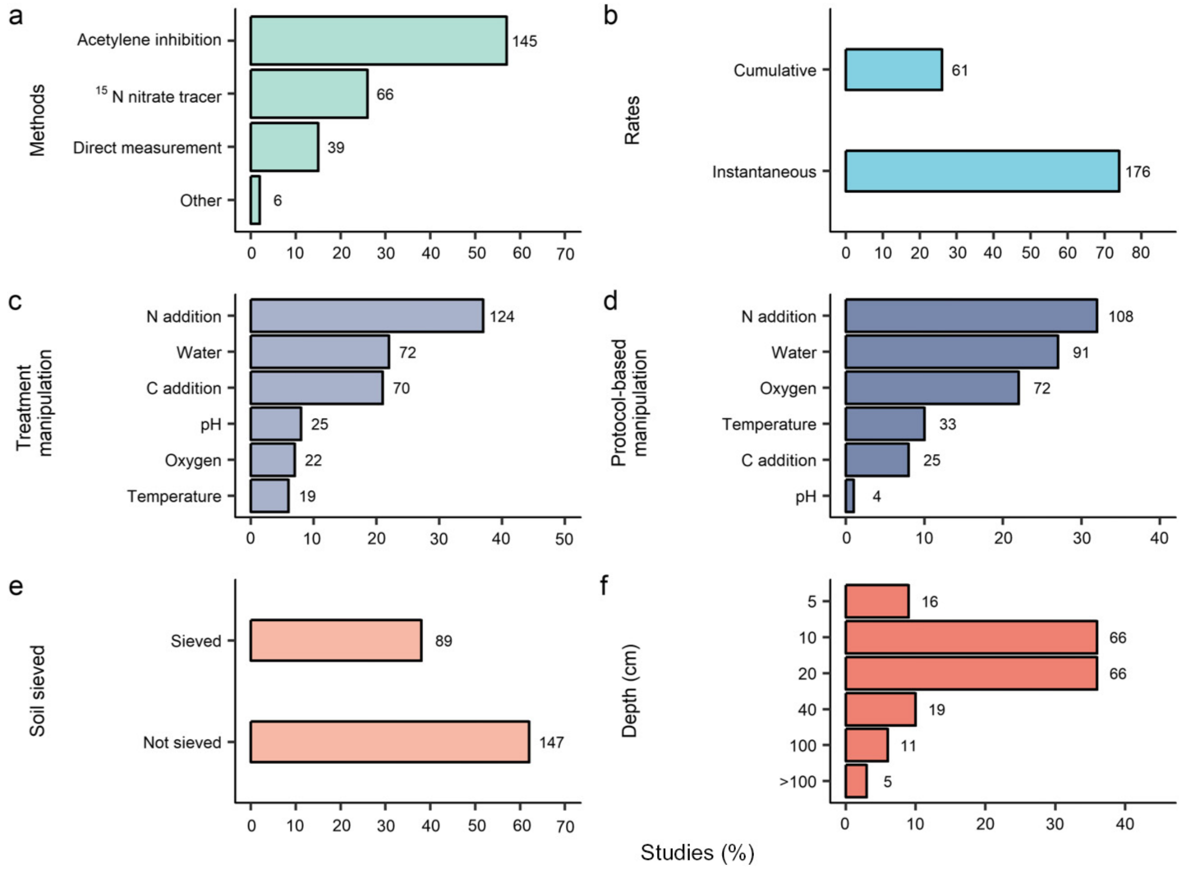

FIG. 2. Census of metadata from upland soil denitrification studies from 1975 to 2015: (a) method used for measuring N_2 production rates, (b) cumulative vs. instantaneous rates reported, (c) treatment manipulation (i.e., imposed only on experimental treatment samples to test for treatment effects), (d) protocol-based manipulation (i.e., imposed on all samples as part of the protocol used to measure N_2 production), (e) whether or not soil samples were sieved, and (f) maximum soil depth measured. Bars represent the percentage of all studies; numbers to the right of each bar indicate the total number of studies.

Most laboratory studies (81%) have focused on surface soils (0–20 cm depth; Fig. 1d) because soil organic C, NO_3^-, denitrifier abundance, and denitrification potential decrease exponentially with soil depth (Uksa et al. 2014, Yang and Silver 2016a, Chen et al. 2018). Although subsoils may exhibit substantially lower denitrification rates, when integrated over a larger mass compared to surface soils, they may account for a large proportion of ecosystem-scale denitrification (van Cleemput 1998, Jahangir et al. 2012). This could potentially explain the absence of groundwater NO_3^- contamination in some agricultural regions (Yu et al. 2019). In addition, depth variation in microbial community composition, including in the diversity of denitrifiers, could lead to differential controls on denitrification in surface vs. subsoil horizons (Uksa et al. 2014, Barrett et al. 2016). Therefore, there is a need for more research emphasis on subsoil denitrification to determine its importance to ecosystem N loss to characterize controls on process rates at depth.

Most studies involved soil manipulation as an experimental treatment or as part of the protocol for measuring soil N_2 production rates. The enzymes responsible for the different steps in denitrification experience transient responses to changes in soil conditions (Firestone et al. 1980, Letey et al. 1980b). Therefore, the timing of sampling after the manipulation is imposed and the study duration greatly influence how the role of the potential controlling variable is deduced from instantaneous rates and cumulative rates, respectively. To better assess controlling variables on denitrification, denitrification rates should be measured on collections of unmanipulated soils that naturally exhibit wide ranges in independent variables such as C, N, soil moisture, and pH. This necessitates researchers selecting methodologies that do not require manipulating these variables to measure soil N_2 production rates, particularly for studies across natural gradients (Appendix S1: Table S1).

Few studies have been conducted in the tropics where the contribution of denitrification to ecosystem N loss is poorly constrained by mass balance estimates. Instead the majority of studies have been concentrated in semiarid and subhumid temperate ecosystems in North

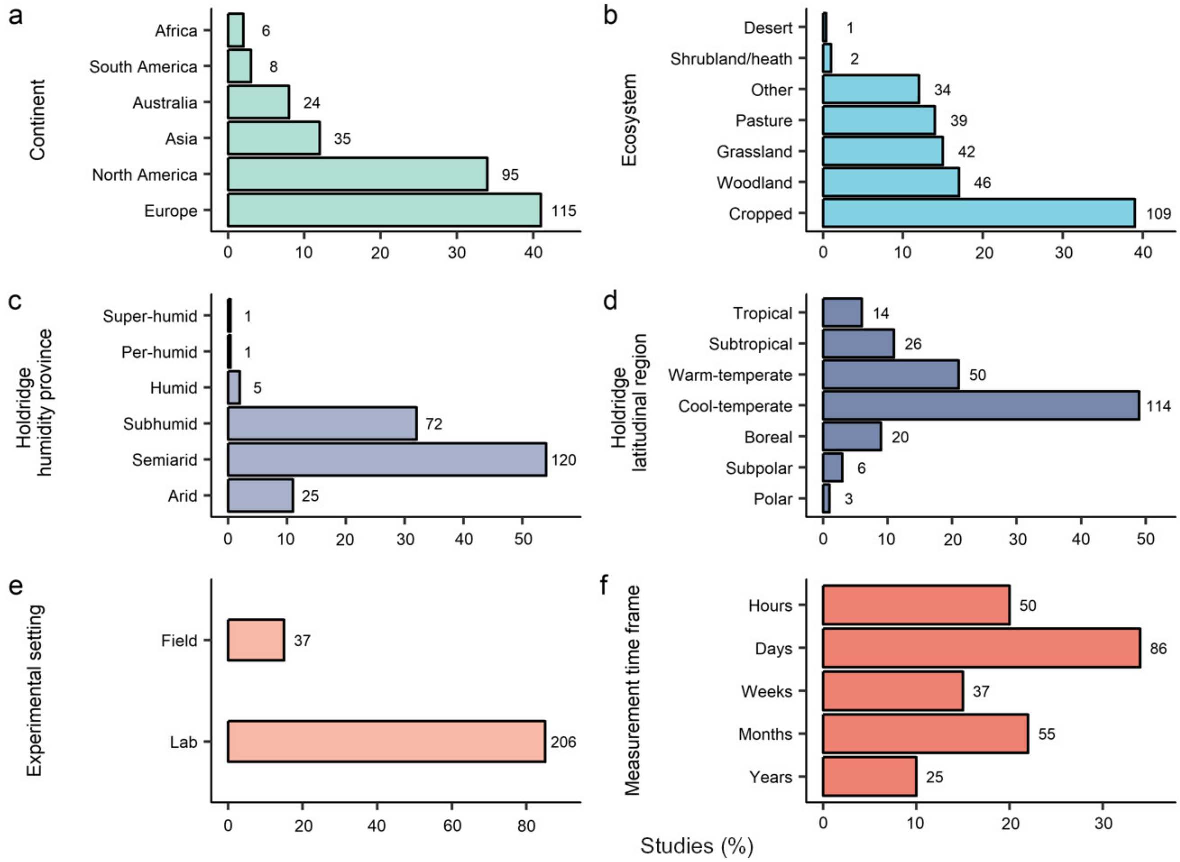

FIG. 3. Census of metadata describing the spatiotemporal context of upland soil denitrification studies from 1975 to 2015: (a) continent on which study site was located, (b) ecosystem type, (c, d) climate based on Holdridge life zones (mean annual temperature and precipitation), (e) experimental context (laboratory vs. field), and (f) measurement time frame. Bars represent the percentage of all studies; numbers to the right of each bar indicate the total number of studies.

America and Europe where high fertilizer inputs support agricultural productivity and stimulate denitrification (Hofstra and Bouwman 2005). We speculate that logistical challenges in conducting research in the tropics combined with the expectation of relatively low denitrification rates in natural ecosystems have led to the low number of tropical studies. However, the warm temperatures, high precipitation, and rapid N cycling that characterize the humid tropics would presumably lead to high denitrification rates. In fact, numerous modeling studies have suggested large N emissions from the tropics (Houlton et al. 2006, Zhuang et al. 2012, Brookshire et al. 2017). With agricultural intensification occurring at a fast rate in the tropics (Lewis et al. 2015), denitrification will play an even more important role in N cycling in these regions, thus it will become increasingly critical to improve estimates of tropical denitrification rates.

In addition to research gaps, we found a lack of standardization across studies that limits our ability to synthesize knowledge from the body of soil denitrification literature. Most importantly, we observed that "denitrification rate" is a term often applied to measurements of net N_2O fluxes and denitrification potential, which are used as proxies for denitrification rates. However, net N_2O fluxes provide no insight into total denitrification rates because N_2O yield (i.e., $N_2O/(N_2 + N_2O)$) varies over the entire range from 0 to 1 in terrestrial ecosystems (Schlesinger 2009). Likewise, denitrification potential, measured in soil slurries under anaerobic conditions with an abundant supply of NO_3^- and glucose, likely does not reflect denitrification rates in the environment (Yeomans et al. 1992). The conflation of net N_2O fluxes and denitrification potential with N_2O and N_2 production rates clouds our ability to discern controls on actual denitrification rates. We also observed that metadata providing potentially important environmental context for interpreting experimental results were variably reported across studies. For example, antecedent soil moisture can affect the response of denitrification to changes in soil moisture (Letey et al. 1980a, Bergstermann et al. 2011). The topographic position from which soils were collected can also mediate the response of denitrification to changes in soil moisture (Krichels et al.

TABLE 2. Comparison of empirical methods used to measure soil N_2O and N_2 production.

Method	Strengths	Weaknesses	Recommended applications	Caution in data interpretation
Acetylene inhibition. Estimates N_2 production as the difference between N_2O production in the absence and presence of acetylene, which inhibits N_2O reduction to N_2 (Yoshinari and Knowles 1976)	Targets N_2 production from denitrification; has high throughput so can include more samples in experimental design; is broadly accessible because requires only a gas chromatograph, low cost, and easy to learn.	Can estimate negative N_2 production rates due to soil heterogeneity between control and acetylene-treated samples; has limited in situ capability.	Comparisons of instantaneous fluxes among sites or experimental treatments.	Measured N_2 production rates are likely underestimates due to acetylene inhibition of nitrification and incomplete inhibition of N_2O reduction by acetylene (Knowles 1990); differences in soil texture or moisture among samples can affect acetylene diffusion, leading to variability and/or bias in measured rates (Knowles 1990).
Direct measurement (i.e., helium gas flow incubation systems). Measures N_2O and N_2 production from intact soil cores incubated under an N_2-free headspace (Butterbach-Bahl et al. 2002)	Directly measures N_2 production as opposed to estimating rates from indirect measurements; measures N_2O and N_2 production from the same soil core to enable accurate estimate of N_2O yield; has low detection limit; does not require addition of substrates or inhibitors.	Has low throughput; requires custom instrumentation; cannot be used to measure in situ rates.	Comparisons of instantaneous fluxes among sites or experimental treatments.	N_2 and N_2O production cannot be attributed solely to denitrification because the method does not partition sources of N_2 and N_2O (e.g., anammox or Feammox could contribute to N_2 production; nitrifier denitrification or chemodenitrification could contribute to N_2O production); measured rates may overestimate N_2O relative to N_2 production due to high surface area exposure to an aerobic headspace.
^{15}N-NO_3 tracer. Measures $^{15}N_2O$ and $^{15}N_2$ production rates by tracing ^{15}N label from soil NO_3^- pool into the N_2O and N_2 pools (Hauck and Melsted 1956)	Targets N_2O and N_2 production from denitrification.	Has low throughput; has high cost for ^{15}N label; requires an isotope ratio mass spectrometer; has limited in situ capability due to requirement for homogenous ^{15}N label distribution.	Experiments in N-rich environments, such as fertilized agricultural fields.	Measured rates may be overestimates because ^{15}N label addition may stimulate process rates, especially in environments with low background NO_3^- (Yang et al. 2014); measured rates may be biased if ^{15}N label is not homogenously distributed (Boast et al. 1988).
^{15}N-N_2O pool dilution. Estimates gross N_2O emission and uptake rates from the isotopic dilution and disappearance of added ^{15}N-N_2O, respectively (Yang et al. 2011)	Can be used for in situ measurements in the field; targets N_2O reduction to N_2 by denitrification.	Has low throughput; requires an isotope ratio mass spectrometer interfaced with a trace gas preconcentration unit for sample analysis; has high cost for ^{15}N-N_2O gas.	Field measurements using surface flux chambers to obtain in situ estimates; experiments in which soils are not flooded, thereby facilitating gas exchange between the chamber headspace and soil pores (e.g., ^{15}N-N_2O diffusion into the soil).	Estimated gross N_2O uptake rates cannot be equated with N_2 production rates because of unknown N_2 production occurring in isolated soil microsites (Wen et al. 2016); in a field setting, an unknown depth of the soil profile is probed by this method (Yang et al. 2011).
N_2:Ar. Estimates N_2 production rates from changes in the N_2:Ar ratio in the headspace of a surface flux chamber or from soil depth profiles of N_2:Ar ratios (Yang and Silver 2012, Fox et al. 2014)	Can be used to measure in situ rates; does not require addition of substrates or inhibitors.	Does not target N_2 production from denitrification; has high detection limit; requires a dual inlet isotope ratio mass spectrometer and vacuum line for gas purification for high precision N_2:Ar analysis, or a membrane inlet mass spectrometer.	Currently not recommended for upland soils due to high detection limit (Yang and Silver 2012).	

TABLE 2. (Continued)

Method	Strengths	Weaknesses	Recommended applications	Caution in data interpretation
Clumped isotopes of N_2. Estimates N_2 production rates based on soil depth profiles of Δ_{30} values representing the proportional deviation in $^{15}N^{15}N$ abundance from a random distribution of ^{14}N and ^{15}N isotopes in N_2 (Yeung et al. 2019)	Can be used to measure in situ rates; does not require addition of substrates or inhibitors.	Does not target N_2 production from denitrification; requires costly ultra-high resolution isotope ratio mass spectrometer for clumped isotope analyses.	Field measurements using soil depth profiles to obtain in situ estimates.	Estimated N_2 production rates depend on the assumptions used to estimate rates from soil depth profiles of Δ_{30}; this new method has not yet been evaluated across soil and ecosystem types, so potential biases and artifacts are not yet fully understood.

2019), possibly due to differences in soil C availability or microbial community composition (Suriyavirun et al. 2019). The use of more precise terminology for measured process rates along with standard reporting of metadata (Table 2) will help resolve these issues to enable future synthesis.

Looking ahead

Here we summarize the research gaps and issues we identified from our census and present specific recommendations to address them:

1. New and improved methods for denitrification have not yet been widely adopted by the scientific community, hindering our understanding of field rates of denitrification and associated controls. The establishment of denitrification research centers for collaborative science or as contract laboratories will increase access to the instrumentation required for using improved methodologies, thereby facilitating their broader use. Given that no method is perfectly suited for addressing all denitrification-related questions (Appendix S1: Table 1), researchers should ideally use a combination of methods complementary in their strengths and weaknesses, and also consider how the weaknesses affect data interpretation (Table 1).
2. A better understanding of how denitrification rates vary across the soil profile is needed to more accurately estimate ecosystem N budgets. Future studies should include both surface and subsoil measurements to better constrain the contribution of subsoils to ecosystem N loss and to determine controls on denitrification in surface soils vs. subsoils.
3. While manipulations can help elucidate drivers of denitrification pulses in response to changes in environmental conditions, the use of unmanipulated controls as well as studies of natural gradients are needed to better understand background patterns in N gas emissions.
4. Tropical soils are generally assumed to have high denitrification rates, yet more data are needed to validate this prediction. International collaborations involving local researchers in the tropics will help overcome logistical challenges of assessing denitrification in tropical systems.
5. The term denitrification rate is often inappropriately used to describe net N_2O fluxes or denitrification potential that do not reflect actual denitrification rates, or to describe N_2O and N_2 gas fluxes that cannot be attributed solely to denitrification. Researchers must use precise terminology or employ methods that target denitrification to avoid confounding denitrification with other processes.
6. The metadata needed to interpret soil denitrification data within the relevant environmental context and to synthesize results across studies are too often excluded from published studies. We suggest that future studies include a standard suite of metadata (Table 2) to facilitate future meta-analyses and synthesis.

A vision for soil denitrification research

The many scientific and logistical challenges faced by soil denitrification researchers require that we work together as a community to overcome these challenges. We envision three paths for coordinating research efforts while recognizing that researchers should retain the flexibility to design experiments for their specific aims. First, at a minimum, researchers should report a standard suite of metadata to facilitate interpretation of their denitrification data within the larger body of literature (Table 2). Second, we propose creating the Global Denitrification Research Network (GDRN) modeled after the Nutrient Network (NutNet), a coordinated research network that facilitates cross-site experiments and syntheses of studies in which data are collected in a consistent manner (Borer et al. 2014). For researchers who want to participate in GDRN, we have provided a minimum protocol to standardize the collection of data from control treatments in experimental studies or collections of soils across natural gradients in observational studies (Appendix S1). This coordination will be critical for elucidating controls on denitrification in unmanipulated soils because syntheses across studies will likely need to span sufficiently wide ranges in controlling variables to determine their hierarchical importance. Third, to facilitate research coordination, we propose forming a consortium of denitrification researchers to facilitate sharing ideas, resources, and to provide mentorship for researchers new to the field. The inclusion of well-instrumented labs as part of the consortium would help support information and technology transfer and increase standardization among research groups. Concurrently pursuing these three paths for coordinated research will help us further advance understanding of soil denitrification.

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Literature Cited

Almaraz, M., E. Bai, C. Wang, J. Trousdell, S. Conley, I. Faloona, and B. Z. Houlton. 2018. Extrapolation of point measurements and fertilizer-only emission factors cannot capture statewide soil NOx emissions. Science. Advances 4: eaau7373.

Barrett, M., M. I. Khalil, M. M. R. Jahangir, C. Lee, L. M. Cardenas, G. Collins, K. G. Richards, and V. O'Flaherty. 2016. Carbon amendment and soil depth affect the distribution and abundance of denitrifiers in agricultural soils. Environmental Science and Pollution Research 23:7899–7910.

Bergstermann, A., L. Cardenas, R. Bol, L. Gilliam, K. Goulding, A. Meijide, D. Scholefield, A. Vallejo, and R. Well. 2011. Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification. Soil Biology & Biochemistry 43:240–250.

Boast, C. W., R. L. Mulvaney, and P. Baveye. 1988. Evaluation of nitrogen-15 tracer techniques for direct measurement of denitrification in soil: I. Theory. Soil Science Society of America Journal 52:1317–1322.

Borer, E. T., W. S. Harpole, P. B. Adler, E. M. Lind, J. L. Orrock, E. W. Seabloom, and M. D. Smith. 2014. Finding generality in ecology: a model for globally distributed experiments. Methods in Ecology and Evolution 5:65–73.

Bouwman, A. F., A. H. W. Beusen, J. Griffioen, J. W. Van Groenigen, M. M. Hefting, O. Oenema, P. J. T. M. Van Puijenbroek, S. Seitzinger, C. P. Slomp, and E. Stehfest. 2013. Global trends and uncertainties in terrestrial denitrification and N_2O emissions. Philosophical Transactions of the Royal Society B 368:20130112.

Boyer, E. W., R. B. Alexander, W. J. Parton, C. S. Li, K. Butterbach-Bahl, S. D. Donner, R. W. Skaggs, and S. J. Del Gross. 2006. Modeling denitrification in terrestrial and aquatic ecosystems at regional scales. Ecological Applications 16:2123–2142.

Brookshire, E. N. J., S. Gerber, W. Greene, R. T. Jones, and S. A. Thomas. 2017. Global bounds on nitrogen gas emissions from humid tropical forests. Geophysical Research Letters 44:2502–2510.

Butterbach-Bahl, K., G. Willibald, and H. Papen. 2002. Soil core method for direct simultaneous determination of N_2 and N_2O emissions from forest soils. Plant and Soil 240:105–116.

Chen, S. M., F. H. Wang, Y. M. Zhang, S. P. Qin, S. C. Wei, S. Q. Wang, C. S. Hu, and B. B. Liu. 2018. Organic carbon availability limiting microbial denitrification in the deep vadose zone. Environmental Microbiology 20:980–992.

Cleveland, C. C., et al. 1999. Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. Global Biogeochemical Cycles 13:623–645.

Davidson, E. A., and W. Kingerlee. 1997. A global inventory of nitric oxide emissions from soils. Nutrient Cycling in Agroecosystems 48:37–50.

Firestone, M. K., and E. A. Davidson. 1989. Microbiological basis of NO and N_2O production and consumption in soil.

Firestone, M. K., M. S. Smith, R. B. Firestone, and J. M. Tiedje. 1979. Influence of nitrate, nitrite, and oxygen on the composition of the gaseous products of denitrification in soil. Soil Science Society of America Journal 43:1140–1144.

Firestone, M. K., R. B. Firestone, and J. M. Tiedje. 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. Science 208:749–751.

Fox, R. J., T. R. Fisher, A. B. Gustafson, T. E. Jordan, T. M. Kana, and M. W. Lang. 2014. Searching for the missing nitrogen: biogenic nitrogen gases in groundwater and streams. Journal of Agricultural Science 152:S96–S106.

Galloway, J. N., et al. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226.

Groffman, P. M., M. A. Altabet, J. K. Bohlke, K. Butterbach-Bahl, M. B. David, M. K. Firestone, A. E. Giblin, T. M. Kana, L. P. Nielsen, and M. A. Voytek. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. Ecological Applications 16:2091–2122.

Groffman, P. M., K. Butterbach-Bahl, R. W. Fulweiler, A. J. Gold, J. L. Morse, E. K. Stander, C. Tague, C. Tonitto, and P. Vidon. 2009. Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. Biogeochemistry 93:49–77.

Hauck, R. D., and S. W. Melsted. 1956. Some aspects of the problem of evaluating denitrification in soils. Soil Science Society of America Journal 20:361–364.

Hedin, L. O., E. N. J. Brookshire, D. N. L. Menge, and A. R. Barron. 2009. The nitrogen paradox in tropical forest ecosystems. Annual Review of Ecology, Evolution, and Systematics 40:613–635.

Hofstra, N., and A. F. Bouwman. 2005. Denitrification in agricultural soils: Summarizing published data and estimating global annual rates. Nutrient Cycling in Agroecosystems 72:267–278.

Holdridge, L. R. 1967. Lie zone ecology. Tropical Science Center, San Jose, Costa Rica.

Houlton, B. Z., D. M. Sigman, and L. O. Hedin. 2006. Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. Proceedings of the National Academy of Sciences USA 103:8745–8750.

Houlton, B. Z., Y. P. Wang, P. M. Vitousek, and C. B. Field. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454:327–U334.

Jahangir, M. M. R., M. I. Khalil, P. Johnston, L. M. Cardenas, D. J. Hatch, M. Butler, M. Barrett, V. O'Flaherty, and K. G. Richards. 2012. Denitrification potential in subsoils: a mechanism to reduce nitrate leaching to groundwater. Agriculture Ecosystems & Environment 147:13–23.

Knowles, R. 1990. Acetylene inhibition technique: development, advantages, and potential problems. Plenum Press, New York, New York, USA.

Krichels, A., E. H. DeLucia, R. Sanford, J. Chee-Sanford, and W. H. Yang. 2019. Historical soil drainage mediates the response of soil greenhouse gas emissions to intense precipitation events. Biogeochemistry 142:425–442.

Letey, J., A. Hadas, N. Valoras, and D. D. Focht. 1980*a*. Effect of pre-incubation treatments on the ratio of N_2O/N_2 evolution. Journal of Environmental Quality 9:232–235.

Letey, J., N. Valoras, A. Hadas, and D. D. Focht. 1980*b*. Effect of air-filled porosity, nitrate concentration, and time on the ratio of N_2O/N_2 evolution during denitrification. Journal of Environmental Quality 9:227–231.

Lewis, S. L., D. P. Edwards, and D. Galbraith. 2015. Increasing human dominance of tropical forests. Science 349:827–832.

McClain, M. E., et al. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301–312.

Schlesinger, W. H.. 2009. On the fate of anthropogenic nitrogen. Proceedings of the National Academy of Sciences USA 106:203–208.

Seitzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications 16:2064–2090.

Sgouridis, F., A. Stott, and S. Ullah. 2016. Application of the ^{15}N gas-flux method for measuring in situ N_2 and N_2O fluxes due to denitrification in natural and semi-natural terrestrial ecosystems and comparison with the acetylene inhibition technique. Biogeosciences 13:1821–1835.

Suriyavirun, N., A. H. Krichels, A. D. Kent, and W. H. Yang. 2019. Microtopographic differences in soil properties and microbial community composition at the field scale. Soil Biology & Biochemistry 131:71–80.

Uksa, M., D. Fischer, G. Welzl, T. Kautz, U. Kopke, and M. Schloter. 2014. Community structure of prokaryotes and their functional potential in subsoils is more affected by spatial heterogeneity than by temporal variations. Soil Biology & Biochemistry 75:197–201.0

van Cleemput, O. 1998. Subsoils: chemo- and biological denitrification, N_2O and N_2 emissions. Nutrient Cycling in Agroecosystems 52:187–194.

Voorhees, E. B. 1902. Studies in denitrification. Journal of the American Chemical Society 24:785–804.

Wang, C., B. Z. Houlton, W. W. Dai, and E. Bai. 2017. Growth in the global N_2 sink attributed to N fertilizer inputs over 1860 to 2000. Science of the Total Environment 574:1044–1053.

Weier, K. L., J. W. Doran, J. F. Power, and D. T. Walters. 1993. Denitrification and the dinitrogen:nitrous oxide ratio as affected by soil water, available carbon, and nitrate. Soil Science Society of America Journal 57:66–72.

Well, R. 2013. Comments on "A test of a field-based 15N-nitrous oxide pool dilution technique to measure gross N_2,O production in soil" by Yang et al. (2011), Global Change Biology, 17, 3577–3588. Global Change Biology 19:133–135.

Wen, Y., Z. Chen, M. Dannenmann, A. Carminati, G. Willibald, R. Kiese, B. Wolf, E. Veldkamp, K. Butterbach-Bahl, and M. D. Corre. 2016. Disentangling gross N_2O production and consumption in soil. Scientific Reports 6:8.

Wen, Y., M. D. Corre, W. Schrell, and E. Veldkamp. 2017. Gross N_2O emission and gross N_2O uptake in soils under temperate spruce and beech forests. Soil Biology & Biochemistry 112:228–236.

Yang, W. H., and W. L. Silver. 2012. Application of the N_2/Ar technique to measuring soil-atmosphere N_2 fluxes. Rapid Communications in Mass Spectrometry 26:449–459.

Yang, W. H., and W. L. Silver. 2016*a*. Gross nitrous oxide production drives net nitrous oxide fluxes across a salt marsh landscape. Global Change Biology 22:2228–2237.

Yang, W. H., and W. L. Silver. 2016*b*. Net soil-atmosphere fluxes mask patterns in gross production and consumption of nitrous oxide and methane in a managed ecosystem. Biogeosciences 13:1705–1715.

Yang, W. H., Y. A. Teh, and W. L. Silver. 2011. A test of a field-based ^{15}N-nitrous oxide pool dilution technique to measure gross N_2O production in soil. Global Change Biology 17:3577–3588.

Yang, W. H., Y. A. Teh, and W. L. Silver. 2013. Measuring gross N_2O production in soil: a reply to Well and Butterbach-Bahl. Global Change Biology 19:985–987.

Yang, W. H., A. C. McDowell, P. D. Brooks, and W. L. Silver. 2014. New high precision approach for measuring ^{15}N–N_2 gas fluxes from terrestrial ecosystems. Soil Biology & Biochemistry 69:234–241.

Yeomans, J. C., J. M. Bremner, and G. W. McCarty. 1992. Denitrification capacity and denitrification potential of subsurface soils. Communications in Soil Science and Plant Analysis 23:919–927.

Yeung, L. Y., J. A. Haslun, N. E. Ostrom, T. Sun, E. D. Young, M. Van Kessel, S. Lucker, and M. S. M. Jetten. 2019. In situ quantification of biological N_2 production using naturally occurring $^{15}N^{15}N$. Environmental Science & Technology 53:5168–5175.

Yoshinari, T., and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications 69:705–710.

Yu, L. F., J. Mulder, J. Zhu, X. S. Zhang, Z. W. Wang, and P. Dorsch. 2019. Denitrification as a major regional nitrogen sink in subtropical forest catchments: Evidence from multi-site dual nitrate isotopes. Global Change Biology 25:1765–1778.

Zhuang, Q. L., Y. Y. Lu, and M. Chen. 2012. An inventory of global N_2O emissions from the soils of natural terrestrial ecosystems. Atmospheric Environment 47:66–75.

Supporting Information

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Data Availability

Data are available from Dryad: https://doi.org/10.25349/D9V30B

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