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A global synthesis of soil denitrification: driving factors and mitigation strategies

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Abstract

Dinitrogen (N_2) and nitrous oxide (N_2O) produced via denitrification may represent major nitrogen (N) loss in terrestrial ecosystems. A global assessment of soil denitrification rate, $N_2O/(N_2O+N_2)$ ratio, and their driving factors and mitigation strategies is lacking. We conducted a global synthesis using 225 studies (3367 observations) to fill this knowledge gap. We found that daily N loss through soil denitrification varied with ecosystems and averaged 0.25 kg N ha^{-1}. The average emission factor of denitrification (EF_D) was 4.8%. The average $N_2O/(N_2O+N_2)$ ratio from soil denitrification was 0.33. Soil denitrification rate was positively related to soil water-filled pore space (WFPS) ($p<0.01$), nitrate (NO_3^-) content ($p<0.05$) and soil temperature ($p<0.01$), and decreased with higher soil oxygen content ($p<0.01$). N_2 emissions increased with latitude ($p<0.05$), WFPS ($p<0.01$) and soil mineral N ($p<0.05$) but decreased with soil oxygen content ($p<0.05$). The $N_2O/(N_2O+N_2)$ ratio increased with soil oxygen content ($p<0.01$) but decreased with organic carbon (C) ($p<0.05$), C/N ratio ($p<0.01$), soil pH ($p<0.05$) and WFPS ($p<0.01$). We also found that optimizing N application rates, using ammonium-based fertilizers compared to nitrate-based fertilizers, biochar amendment, and application of nitrification inhibitors could effectively reduce soil denitrification rate and associated N_2 emissions. These findings highlight that N loss via soil denitrification and N_2 emissions cannot be neglected, and that mitigation strategies should be adopted to reduce N loss and improve N use efficiency. Our study presents a comprehensive data synthesis for large-scale estimations of denitrification and the refinement of relevant parameters used in the submodels of denitrification in process-based models.

1 Introduction

Denitrification is a microbial process by which nitrate (NO_3^-) is gradually reduced to nitrous oxide (N_2O) and dinitrogen (N_2) (Zaman *et al.*, 2012); it is a major pathway of nitrogen (N) loss in

terrestrial ecosystems. N_2O emissions contribute to global warming and stratospheric ozone depletion whereas N_2 losses decrease nitrogen use efficiency (NUE) in croplands and grasslands (Bouwman *et al.*, 2013). The N loss through soil denitrification from terrestrial ecosystems was estimated to be 105-185 Tg N yr^{-1} (Tiedje, 1988) and 166 Tg N yr^{-1} (Scheer *et al.* (2020), with around 66-87 Tg N yr^{-1} in global croplands (Hofstra and Bouwman, 2005; Seitzinger *et al.*, 2006) and 5.6 Tg N yr^{-1} in temperate grasslands (Saggar *et al.*, 2013). N_2 represents a substantial N loss accounting for up to 85% of total denitrification (year 2000) (Bouwman *et al.*, 2013) but it is often overlooked and poorly studied as N_2 is non-reactive (Almaraz *et al.*, 2020). The quantification of N_2 emissions from terrestrial ecosystems remains challenging because of the high background atmospheric N_2 concentration (Zaman *et al.*, 2012; Saggar *et al.*, 2013). Many empirical and process-based models were developed to estimate N_2O and N_2 emissions from denitrification based on a fixed N_2O to N_2 ratio (NOE model) (Hénault *et al.*, 2005) or combined with functions of soil properties (e.g., APSIM and DayCent model) (Del Grosso *et al.*, 2000; Keating *et al.*, 2003; Stehfest and Muller, 2004). However, the N_2O and N_2 emissions from soil denitrification showed a large spatial and temporal heterogeneity at a global scale (Butterbach-Bahl and Dannenmann, 2011). Therefore, a better understanding of the $N_2O/(N_2O+N_2)$ ratio and its driving factors could improve the estimation of denitrification in earth system models (Almaraz *et al.*, 2020).

Soil denitrification and the emissions of its end-products (N_2O and N_2) are regulated by soil and environmental factors. For example, soil NO_3^- availability affects the denitrification rate (Abbasi and Adams, 1999; Senbayram *et al.*, 2019). Higher carbon (C) availability could increase the denitrification rate but decrease the N_2O/N_2 ratio (Clough *et al.*, 1998; Gillam *et al.*, 2008). The effect of soil pH on denitrification is controversial. It is generally accepted that acidic soil has a lower soil denitrification rate (Sun *et al.*, 2012) and higher $N_2O/(N_2O+N_2)$ ratio (Zaman *et al.*,

2007; Zaman and Nguyen, 2010). Soil pH can also indirectly affect denitrification because nitrification is inhibited in acidic soils and subsequently less NO_3^- is available for denitrification (Tiedje, 1988). On the contrary, Šimek *et al.* (2000) found no significant correlation between soil pH and denitrifying enzyme activity. Soil oxygen concentration, water content and rainfall are other important factors as anaerobic conditions favour denitrification (De Klein and Van Logtestijn, 1996; Ellis *et al.*, 1998; Rudaz *et al.*, 1999; Hefting *et al.*, 2003; Saggar *et al.*, 2004). Generally, soil temperature is positively related to soil denitrification rate and associated N_2O emissions (Knowles, 1982; Ryden, 1983). However, some studies demonstrated that N_2O emissions may decrease with increasing temperature, because elevated temperature may enhance anaerobiosis and decrease the N_2O/N_2 ratio (Butterbach-Bahl and Dannenmann, 2011). These inconsistencies highlight the need of a comprehensive assessment to identify the important drivers of soil denitrification rate and its end-products.

Mitigation strategies have been widely investigated and adopted to lower soil denitrification and increase NUE. Optimizing N application rate could reduce the excess N that is subjected to denitrification (Morley *et al.*, 2014; Lai and Denton, 2018; Senbayram *et al.*, 2019). The application of soil amendments e.g., lime (McMillan *et al.*, 2016) and biochar (Cayuela *et al.*, 2013) generally raised soil pH and increased the activities of N_2O reductase that might subsequently decrease N_2O emissions from denitrification. Reduced crop residue retention decreases organic C input for soil denitrifiers (Chintala *et al.*, 2015; Sun *et al.*, 2018) but may reduce the potential of C sequestration and affect crop yield (Xia *et al.*, 2018). Avoiding excessive irrigation could effectively maintain oxygen supply in the soil and lower denitrification (Huang *et al.*, 2015). The application of nitrification inhibitors can reduce the NO_3^- availability for denitrification by inhibiting nitrification without impairing crop yields (McGeough *et al.*, 2012;

87 Wang *et al.*, 2017). A systematic synthesis of mitigation strategies for denitrification and N_2 is
88 vital for improving global fertilizer NUE and environmental quality.
89 In this study, we compiled a comprehensive database based on peer-reviewed studies worldwide.
90 We attempted to 1) summarize and quantify soil denitrification rate, N_2 emissions and the
91 $N_2O/(N_2O+N_2)$ ratio across different land use systems and climatic zones; 2) quantify the
92 relationships between soil/environmental factors and soil denitrification rate, N_2 emissions and the
93 $N_2O/(N_2O+N_2)$ ratio; and 3) evaluate the effects of mitigation strategies on soil denitrification rate
94 and N_2 production.

95 **2 Data and Methods**

96 **2.1 Database compilation**

97 An extensive keyword search was conducted from Web of Science (ISI), CAB Abstracts (ISI) and
98 Google Scholar between 1978 and 2019. The keywords used for searching include the
99 combinations of denitrification, N_2/dinitrogen, nitrogen (N) application, mitigation strategy,
100 management practices, fertilizer type, soil amendment and inhibitor, $N_2O/(N_2O+N_2)$ ratio. The
101 database was also cross-checked with the studies included in recent key review papers of
102 denitrification by Wang *et al.* (2018) and Almaraz *et al.* (2020). These papers did not examine the
103 aspects of land use types or mitigation strategies of soil denitrification and associated gaseous
104 losses. Literature included in our database fulfilled the following selection criteria: a) soil
105 denitrification rate and its N_2O or N_2 emissions in the topsoil (0-20 cm) were specified; b)
106 experimental design or lab incubation conditions were given; c) studies were restricted to terrestrial
107 ecosystems. After data collection, a total of 3367 observations (1599 observations for soil
108 denitrification rate, 860 observations for soil N_2 emissions and 908 observations for the
109 $N_2O/(N_2O+N_2)$ ratio from soil denitrification) were included in the database.

In the database, geographic coordinates (latitude and longitude) of the study sites, environmental conditions (mean annual temperature [MAT] and mean annual precipitation [MAP]), soil physicochemical properties (e.g., soil texture, clay content, soil water filled pore space (WFPS), soil temperature under incubation condition, soil pH, soil total nitrogen (TN) and organic carbon (SOC) content, soil C/N ratio, NH_4^+ and NO_3^- concentrations at the beginning of the experiment, etc.), and management practices (N application rate and farming practices) were extracted. This database was used for conducting linear mixed-effect models (section 3.2) and a meta-analysis (section 3.3). To decrease the outliers' impact and increase the normality, some data were natural log transformed when necessary for statistical analysis.

The database covered the dominant vegetation types (e.g., upland fields, paddy fields, grasslands, forests and wetlands). Overall, these sites spanned from 123.27°W to 176.07°E and from 42.97°S to 60.62°N (Figure 1). These sites were classified into four major climatic zones according to Köppen Climate Classification (tropical, dry, temperate, and continental climates).

2.2 Data analyses

We calculated the emission factor of denitrification (EF_D) from field experiments conducted for at least a cropping season using the following equation:

$$EF_D(\%) = \left(\frac{\text{cumulative denitrification N loss } (N_2O+N_2)_{\text{ treatment}} - \text{cumulative denitrification N loss } ((N_2O+N_2))_{\text{control}} \text{ (kg N ha}^{-1})}{\text{N fertilizer/manure applied ((kg N ha}^{-1})}\right) * 100\ (\%) \quad \text{.....(1)}$$

We then used linear mixed-effect models to explore the relationships between soil denitrification rate, soil N_2 emissions and the $N_2O/(N_2O+N_2)$ ratio from denitrification with soil and environmental properties:

$$\ln y = \alpha + \beta x + T_{study} + \varepsilon \quad (2)$$

Where *y* is soil denitrification rate and should be natural log transformed to satisfy the assumption of normality in the context of the model, soil N_2 emissions or the $N_2O/(N_2O+N_2)$ ratio from denitrification. α, β and ε are the intercept, slope and random error, respectively; *x* is the soil WFPS, soil O_2 concentration, SOC, C/N ratio, NO_3^- concentration, soil temperature, soil mineral N content, soil pH, N application rate or latitude. T is the random effect factor. We considered 'study' as a random effect to reflect the non-independence among observations within the same experiment. The linear mixed-effect model was performed using *lme4* package (Bates *et al.*, 2014) and *lmerTest* package (Kuznetsova *et al.*, 2017). The average and median soil denitrification rate, EF_D and $N_2O/(N_2O+N_2)$ ratio from denitrification were calculated. Tukey HSD test was performed across land uses and climatic zones by *agricolae* package. (Mendiburu, 2014). These analyses were conducted in R (RStudio, 2020).

We also used paired observations to perform a meta-analysis to examine the effects of mitigation strategies on denitrification and N_2 emissions using our database. For N application, we used zero or the lowest N as the control and the other N application rates as treatments. For fertilizer type, we considered urea, a widely used fertilizer, as the control and other fertilizer types as treatments. Fertilizer types were sub-divided into organic fertilizers (animal manure and solid waste), ammonium-based fertilizers, nitrate-based fertilizers and NH_4NO_3 fertilizer. For management practices, soil amendment (biochar, zeolite and lime) or the use of nitrification inhibitor was compared to N fertilization only. We also considered no tillage, no irrigation, residue removal, single fertilization and surface application of fertilizers as controls, and compared them with their corresponding treatments: tillage, irrigation, residue retention, split fertilization and incorporation of fertilizers.

Effect sizes in meta-analysis were weighed by the number of replicates in a study (Lam *et al.*, 2012) to avoid extreme weights:

$$\text{Weight} = (n_c \times n_T)/(n_c + n_T) \quad (3)$$

Where n_c and n_T are the number of replicates of the control and treatment, respectively.

The response ratio *r* is represented by the natural log and used as a metric for soil denitrification rate and N_2 emissions in response to management practices (Hedges *et al.*, 1999):

$$\ln r = \ln\left(\frac{\overline{x}^T}{\overline{x}^C}\right) \quad (4)$$

where $\overline{x}^T$ and $\overline{x}^C$ are the mean values of the treatment observation and the control observation, respectively. Results of meta-analysis are reported as the percentage change of soil denitrification and N_2 emissions under treatment effects ((*r*-1) ×100). Fixed-effect meta-analysis with 4999 iterations bootstrapping was conducted to generate the mean effect size and the 95% confidence intervals (CIs) by MetaWin version 2.1 (Rosenberg *et al.*, 2000). The effects of mitigation strategies were considered significant if the 95% CIs did not overlap with zero.

3 Results

3.1 Summary of soil denitrification and N_2 emissions studies

The summarized data from 648 field observations suggested a mean daily loss of 0.25 kg N ha^{-1} (median value of 0.06 kg N ha^{-1}) through soil denitrification (Figure 2). Soil denitrification rate was the highest in wetlands with an average of 0.89 kg N ha^{-1} d^{-1} followed by paddy fields (0.62 kg N ha^{-1} d^{-1}), while upland fields had a lower rate of 0.11 kg N ha^{-1} d^{-1}. In terms of climatic zones, the highest denitrification rate with an average of 0.83 kg N ha^{-1} d^{-1} was found in tropical areas while the lowest rate (0.11 kg N ha^{-1} d^{-1}) in continental areas. The overall EF_D was 4.8% (median value of 2.6%) (Figure 3). Paddy fields had the highest EF_D with an average of 27%, which was

significantly higher than grassland and upland fields (4.7% and 4.1%, respectively). Across climatic zones, temperate regions had the highest mean EF_D (5.2 %), followed by continental and dry areas (3.2% and 1.9%, respectively).

Overall, the mean $N_2O/(N_2O+N_2)$ ratio was 0.33 (Figure 4). The $N_2O/(N_2O+N_2)$ ratio across different ecosystems followed the order of grassland (0.37) > wetland (0.36) > upland fields (0.35) > paddy field (0.23) >forest (0.12), and that across climatic regions the order of continental (0.38) > temperate (0.35) > dry (0.25) > tropical (0.20).

3.2 Bivariate relationships of soil denitrification rate, N_2 emissions and $N_2O/(N_2O+N_2)$ with soil and environmental properties

Soil denitrification rate was significantly increased with soil WFPS ($p<0.001$; Figure 5a) but decreased with increasing O_2 concentration ($p<0.001$; Figure 5b). Soil denitrification rate was positively related to soil NO_3^- content ($p<0.05$; Figure 5c) and mineral N content ($p< 0.05$; Figure 5d). The relationship between soil denitrification and soil pH was not significant (Figure 5e). Soil temperature was positively correlated to soil denitrification rate ($p<0.001$, Figure 5f).

Soil N_2 emissions from denitrification significantly increased from low to high latitude ($p<0.05$; Figure 6a). Soil mineral N and WFPS were positively related to soil N_2 emissions ($p<0.05$ and $p<0.001$, respectively; Figure 6b, c). N_2 emissions decreased significantly with increased O_2 concentration ($p<0.05$; Figure 6d).

The $N_2O/(N_2O+N_2)$ ratio was negatively related to soil pH ($p<0.05$; Figure 7a) and WFPS ($p<0.01$, Figure 7b). The soil C/N ratio and organic C contents were also negatively related to the $N_2O/(N_2O+N_2)$ ratio ($p<0.05$; Figure 7d, f). However, the $N_2O/(N_2O+N_2)$ ratio was positively related to soil O_2 concentration ($p<0.01$; Figure 7c).

3.3 The effect of management practices on soil denitrification rate and N_2 production

Increasing N application rate significantly increased soil denitrification rate by 219% (Figure 8a) and N_2 emissions by 204% (Figure 8b). Under the same N application rate, compared to urea, other types of fertilizer led to higher soil denitrification rate by 77% (Figure 8a). Among these fertilizer types, the application of NO_3^--based fertilizer produced the highest increase in soil denitrification rate (199%) (Figure 8a), whereas applying NH_4-based fertilizer significantly reduced N_2 emissions by 56% (Figure 8b). Lime application significantly increased soil denitrification rate and N_2 emissions by 52% and 48%, respectively (Figure 8a, b), whereas biochar application reduced soil denitrification rate by 38% but had no significant effect on N_2 emissions. The application of zeolite had no significant effects on soil denitrification or N_2 production. Residue retention and irrigation significantly increased soil denitrification rate (144% and 75%, respectively) and N_2 emissions (226% and 102%, respectively) (Figure 8a, b). Tillage, split application and fertilizer incorporation had no significant effects on soil denitrification rate or N_2 emissions (Figure 8a, b). Overall, nitrification inhibitor application significantly reduced soil denitrification rate and N_2 emissions by 41% and 30%, respectively (Figure 8a, b). 3,4-Dimethylpyrazole phosphate (DMPP) was the most effective inhibitor in reducing denitrification rate (71%) and N_2 emissions (68%). Dicyandiamide (DCD) reduced soil denitrification rate but had no significant effect on N_2 emissions. 2-Chloro-6-(trichloromethyl) pyridine (Nitrapyrin) decreased N_2 emissions by 15%.

4 Discussion

4.1 Denitrification is a key N loss pathway globally

Denitrification occurs in a wide range of terrestrial ecosystems. Typically, denitrification requires an anoxic environment with readily available soil NO_3^- and organic C, so fertilized and/or irrigated paddy fields and grasslands located in humid areas had higher denitrification rates (Figure 2).

Moreover, anaerobic microsites within soil aggregates can produce hot-spots and hot moments of denitrification activity, resulting in significant denitrification from apparently well-drained soils (Scheer *et al.*, 2020), such as in the croplands and grasslands in dry climatic areas. The average EF_D calculated in our study was 4.8% and could be up to 41.7% in paddy fields and grasslands in temperate areas (Figure 3). This indicated that better management strategies should be adopted because N loss via denitrification could decrease NUE and plant productivity.

The $N_2O/(N_2O+N_2)$ ratio varied greatly across land use types and climatic zones with a mean value of 0.33, which implies that N_2 emissions dominated the end-products of denitrification. The $N_2O/(N_2O+N_2)$ ratio in denitrification was used to estimate N_2 emissions in process-based models. These models generally assume the ratio is a fixed value (Hénault *et al.*, 2005) or determined by a function based on soil properties such as soil water condition and soil NO_3^- content (Del Grosso *et al.*, 2000; Stehfest and Muller, 2004; Li *et al.*, 2007). In this regard, the wide range of $N_2O/(N_2O+N_2)$ ratio observed in this study suggests that the estimation of N_2 emissions in process-based models is highly uncertain. In fact, the $N_2O/(N_2O+N_2)$ ratio adopted in process-based models was derived from limited datasets and the relationships between N_2 emission and its key drivers might not have been well captured in these models.

4.2 Key factors of soil denitrification rate, N_2 emissions and $N_2O/(N_2O+N_2)$ ratio

We found that N loss via denitrification could be substantial and varied greatly with edaphic and environmental conditions. These results confirm the traditional conceptual frameworks laid out by Tiedje (1988) and Robertson (1989). Meanwhile, a better understanding of the driving factors of denitrification and its end products is vital for developing effective management strategies that mitigate N_2O and N_2 losses and improve NUE.

Soil NO_3^- is the primary N substrate for soil denitrifying bacteria. We found that soil NO_3^- content was positively related to denitrification rate, which has been widely reported (Zaman *et al.*, 2007; Senbayram *et al.*, 2012). According to our database, when soil NO_3^- content reached a high level (80 mg N kg^{-1}), a higher $N_2O/(N_2O+N_2)$ ratio (>0.5) was observed in highly fertilized soils such as croplands and grasslands. This could be attributed to the suppression of the activity of N_2O reductase, the enzyme responsible for reducing N_2O to N_2 (Senbayram *et al.*, 2019), resulting in incomplete denitrification and higher $N_2O/(N_2O+N_2)$ ratio (Blackmer and Bremner, 1978; Stevens and Laughlin, 1998).

With decreased WFPS, the synthesis of denitrifying enzymes and N_2O reductase is relatively inhibited; thus, denitrification is reduced (Hwang and Hanaki, 2000). Our database indicated that when WFPS was over 72%, more N_2 than N_2O was emitted through denitrification, thereby decreasing $N_2O/(N_2O+N_2)$ ratio. The activity of N_2O reductase was decreased with increased soil O_2 concentration (Friedl *et al.*, 2016). At high soil water content, the limited O_2 availability in the soil resulted in anaerobic conditions. Subsequently, N_2O reductase capacity was upregulated, leading to a lower $N_2O/(N_2O+N_2)$ ratio (Figure 7c).

We found that denitrification occurred over a wide range of soil temperatures (2-45 ºC) and was positively related to soil temperature (Figure 5f). Soil respiration increased with soil temperature, resulting in O_2 depletion and anaerobic conditions. This promoted the activity of denitrifiers and denitrification (Lai and Denton, 2018).

Soil pH regulates denitrification and its end products (Dannenmann *et al.*, 2008; Sun *et al.*, 2012). N_2O reductase was less active under low pH (Liu *et al.*, 2010). At a reduced soil pH, denitrification may liberate more N_2O and subsequently increase the ratio of $N_2O/(N_2O+N_2)$ (Simek and Cooper, 2002; Cuhel *et al.*, 2010; Qu *et al.*, 2014). However, the relationship between denitrification and

soil pH was not significant in this study (Figure 5e). This might be attributed to the confounding effects of other factors such as the application of N fertilizers and organic amendments under aerobic conditions in upland fields and grasslands. Under anaerobic conditions (paddy fields and wetlands) soil pH was positively correlated with denitrification (Figure S4).

We found that for a soil with a higher C/N ratio (>14), more N_2O was reduced to N_2, decreasing the $N_2O/(N_2O+N_2)$ ratio from denitrification (Figure 7d). This is because higher soil C/N ratio promoted denitrifying population and the diffusion rate of soil NO_3^- into denitrifying microsites (Saggar *et al.*, 2013). Moreover, soil organic C was highly related to the ratio of N_2O to total denitrification (Figure 7f). More available C could promote the growth of soil microorganisms and increase N_2O reductase in the soil, which could reduce the $N_2O/(N_2O+N_2)$ ratio from denitrification. However, the effect of soil organic C on $N_2O/(N_2O+N_2)$ can be outweighed by other factors, such as soil NO_3^- content (Weier *et al.*, 1993).

4.3 Management practices to mitigate soil denitrification and N_2 emissions

Our meta-analysis showed that optimizing N application rate to match plant N demand and avoiding excessive NO_3^- in the soil can effectively reduce denitrification and N_2 emissions (Figure S1d). The type of N fertilizers can also impact denitrification and N_2 emissions. Compared to urea, NO_3^- based fertilizers and organic fertilizers significantly increased denitrification by 77% (Figure 8) under the same application rate. Nitrate-based fertilizers provide more NO_3^- compared to urea, which is the key substrate for denitrification. We found that soil NO_3^- content was significantly correlated with soil denitrification rate and N_2 emissions (Figure 5c, 6e). Our analysis also indicated that the application of urea/ammonium-based fertilizers could reduce N_2 emissions compared to nitrate-based or organic fertilizers. When nitrate-based fertilizers are applied, more NO_3^- is available for denitrification and subsequent gaseous losses (Wang *et al.*, 2018). Organic

289 fertilizers like livestock manure, increased denitrification by providing the energy source (readily
290 available C) for denitrifiers and aggravate anaerobic conditions through its decomposition (Figure
291 9) (Estavillo *et al.*, 1994; Mogge *et al.*, 1998; Barton *et al.*, 1999).
292 Managing crop residue properly is important to regulate denitrification and N_2 emissions without
293 impairing soil fertility and crop yields. Our results showed that residue retention could increase
294 denitrification and N_2 emissions by 144% and 226%, respectively (Figure 8), because it can
295 increase C availability for denitrifiers and promote subsequent denitrification (Garcia-Montiel *et*
296 *al.*, 2003). In addition, crop residue decomposition consumes O_2 thereby inducing higher
297 denitrification and N_2 emissions (Clough *et al.*, 1998; Gillam *et al.*, 2008) (Figure 9). It is worth
298 nothing that the effect of residue retention on denitrification and N_2 emissions is highly related to
299 residue quality. Incorporating crop residues with low C/N ratios would promote denitrification by
300 providing sufficient N for soil microbial growth and proliferation (Xia *et al.*, 2018). However, the
301 application of crop residue with higher C/N ratios (e.g., wheat straw and switchgrass) could reduce
302 denitrification by promoting soil microbial N immobilization (Sun *et al.*, 2018).
303 Irrigation could temporarily increase soil water content and develop an anaerobic zone that favours
304 denitrification especially for fine-textured soils (De Klein and Van Logtestijn, 1996; Zaman *et al.*,
305 2012; Saggar *et al.*, 2013) (Figure 9). In this regard, advanced irrigation techniques (e.g., drip
306 irrigation) should be adopted to reduce denitrification and associated N_2 emissions (Barakat *et al.*,
307 2016).
308 Biochar application effectively reduced soil denitrification rate by 38% (Figure 8a), because it
309 promoted the chemical affinity of inorganic N substrates (Cayuela *et al.*, 2013; Chintala *et al.*,
310 2015). However, N_2 emissions overall were not significantly affected by biochar application
311 (Figure 8b), likely because biochars have diverse characteristics e.g., pH and C/N ratio, and may

exert contrasting effects on N_2 emissions (Malghani *et al.*, 2018). Lime is often applied in pastures and wetlands to decrease total N_2O emissions (Saggar *et al.*, 2013) but may increase soil denitrification rate and N_2 emissions (Simek and Cooper, 2002). Liming can also increase soil pH and subsequently enhance the activity of N_2O reductase, which reduces N_2O to N_2 in acidic soils. Nitrification inhibitors delay the conversion of NH_4^+ to NO_3^- via nitrification (Zaman *et al.*, 2012) (Figure 9). This delay maintains the applied N fertilizer/animal manure in the form of NH_4^+ limiting NO_3^- availability for denitrification and decreases N_2O and N_2 emissions (McGeough *et al.*, 2012; Maris *et al.*, 2015; Friedl *et al.*, 2017). However, nitrification inhibitors may increase N loss via NH_3 volatilization (Pan *et al.*, 2016; Lam *et al.*, 2017; Xia *et al.*, 2017; Lam *et al.*, 2018). Therefore, additional knowledge-based N managements (e.g., fertilizer deep placement) should be adopted simultaneously with nitrification inhibitors to reduce the overall N losses (Xia *et al.*, 2020).

Many studies have focused on mitigation strategies to reduce potent greenhouse gas N_2O emissions and how to facilitate N_2O reduction to N_2. However, from the perspective of N use efficiency, N loss via N_2 should be minimized. Management practices that can reduce both denitrification and N_2 emissions should be recommended considering the trade-off between N_2O mitigation and N use efficiency improvement. Our meta-analysis suggests that reduced N application rate, use of ammonium-based fertilizers, reduced residue retention, biochar amendment and nitrification inhibitors, and improved irrigation techniques such as drip irrigation could effectively reduce denitrification and N_2 emissions.

5 Uncertainty

We are aware that the number of observations included in the database varies largely across land uses; more relevant studies should be conducted in different ecosystems. Moreover, the acetylene

inhibition technique (AIT) has been widely used but may underestimate denitrification rate (Wang and Yan, 2016) under both field and laboratory conditions (Figure S5) mainly owing to the inhibition of nitrification (Groffman *et al.*, 2006; Qin *et al.*, 2013). Previous studies demonstrated that AIT may underestimate denitrification by 4-10 times compared to ^{15}N tracer method or gas-flow-soil-core techniques when applied to the same soils (Watts and Seitzinger, 2000; Lindau *et al.*, 2011; Sgouridis *et al.*, 2016). Although ^{15}N tracer method or gas flow core techniques could reliably estimate denitrification rate (Groffman *et al.*, 2006), they are not widely adopted, as evidenced by the number of observations in our database (^{15}N-gas flux method: n=268; gas-flow (helium or Ar) method: n=131; AIT: n=1200). Such bias in the observation number among different methods might have caused uncertainties in quantifying soil denitrification rate in this study. Besides, some studies that used Nitrogen-Free Air Recirculation Method (N-FARM) to measure the denitrification rate (Burgin and Groffman, 2012) may overestimate the N_2O from denitrification because it cannot distinguish the N_2O produced from denitrification and that from nitrification. Therefore, more studies using advanced techniques should be conducted. We are also aware that NO emissions are a product of denitrification and some studies applied stable isotope models (Houlton *et al.*, 2006) to estimate NO emission from denitrification. We only identified N_2O and N_2 emissions from denitrification owing to the limited studies on $NO/N_2O/N_2$ ratio.

6 Conclusions

We compiled a global database on denitrification rate and its N_2O and N_2 emissions. We found that N loss through denitrification varied greatly across land uses and climatic regions. The average EF_D was 4.8%. The wide range of $N_2O/(N_2O+N_2)$ demonstrated that the adoption of a fixed ratio in some process-based models for estimating N_2 emissions from denitrification is not suitable. We found that N_2 loss accounted for 67% of total denitrification. N loss as N_2, although harmless to

the environment, deserves more attention from the perspective of improving N use efficiency. Denitrification and its N_2O and N_2 emissions are highly dependent on soil properties, e.g., soil mineral N, soil water, temperature and oxygen conditions. We further examined the potential of mitigation strategies in decreasing denitrification and N_2 emissions. Specifically, optimized N input, the use of ammonium-based fertilizers, biochar amendment, use of nitrification inhibitors, and advanced irrigation technique could effectively decrease denitrification and N_2 emissions.

Acknowledgements

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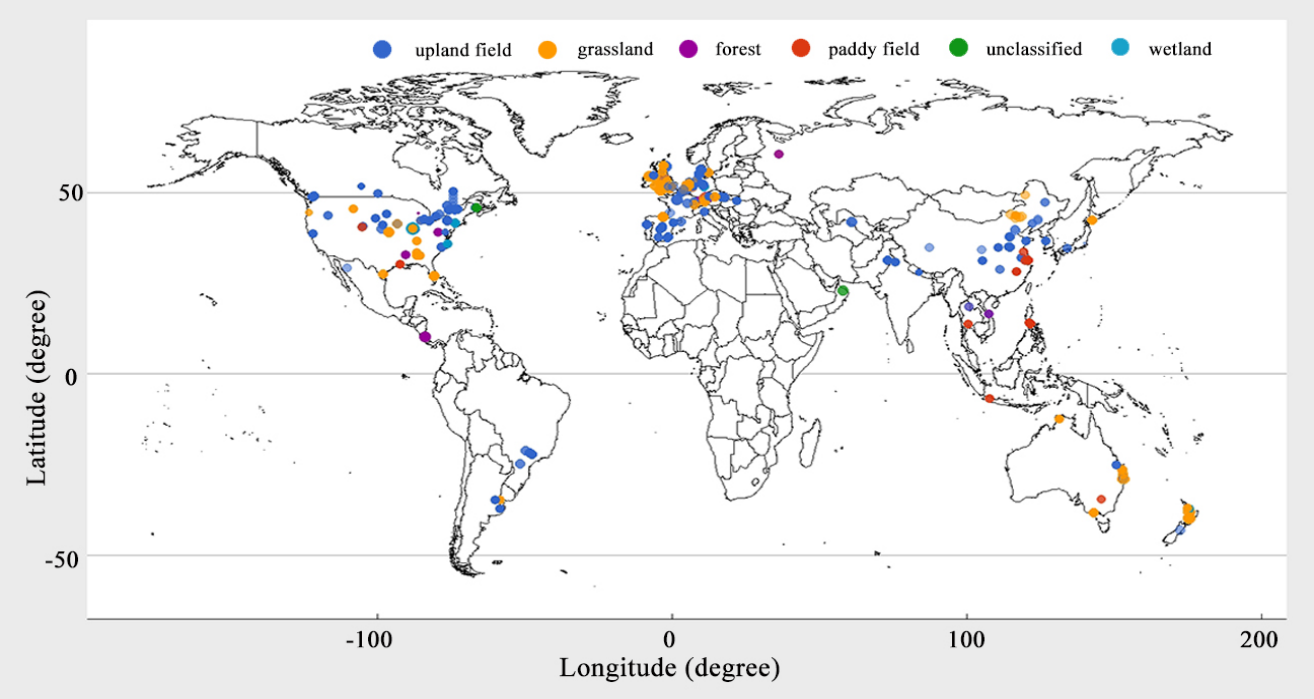

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585 Figure 1. Location of study sites included in the database

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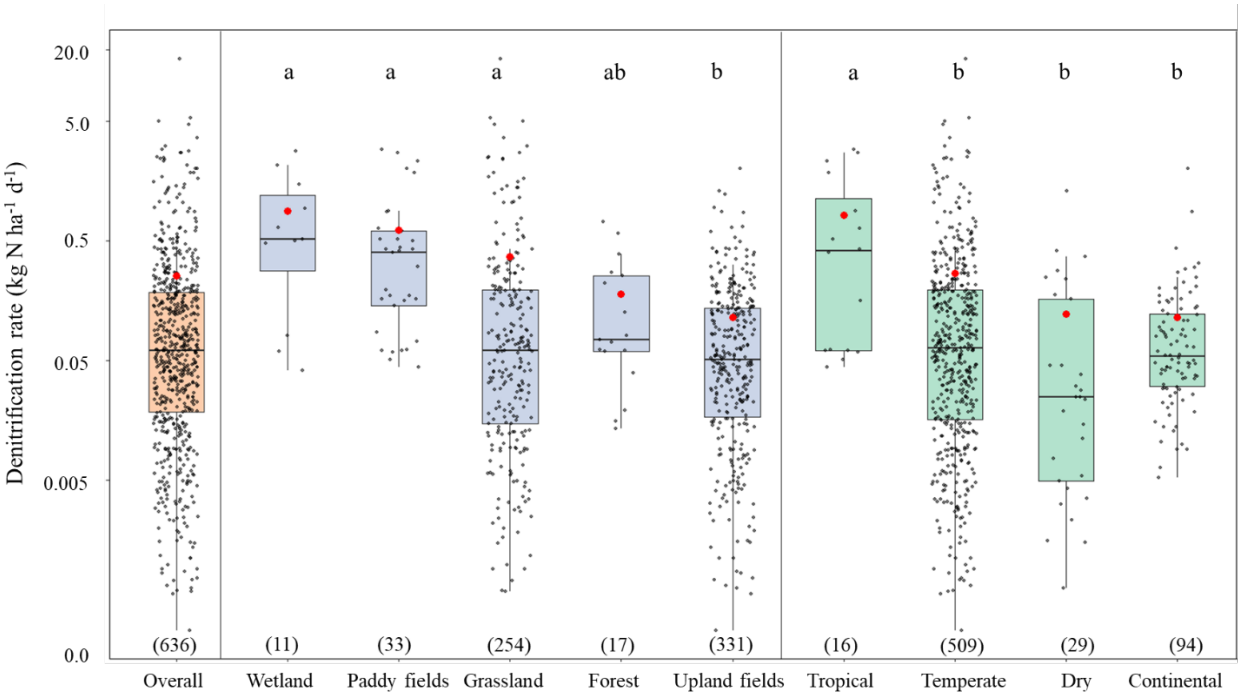

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591 Figure 2. Denitrification rate across land uses and climatic zones from field experiments. Values

592 in parentheses represent the number of observations. Different letters on the top indicate that the

593 mean values are significantly different at $p < 0.05$.

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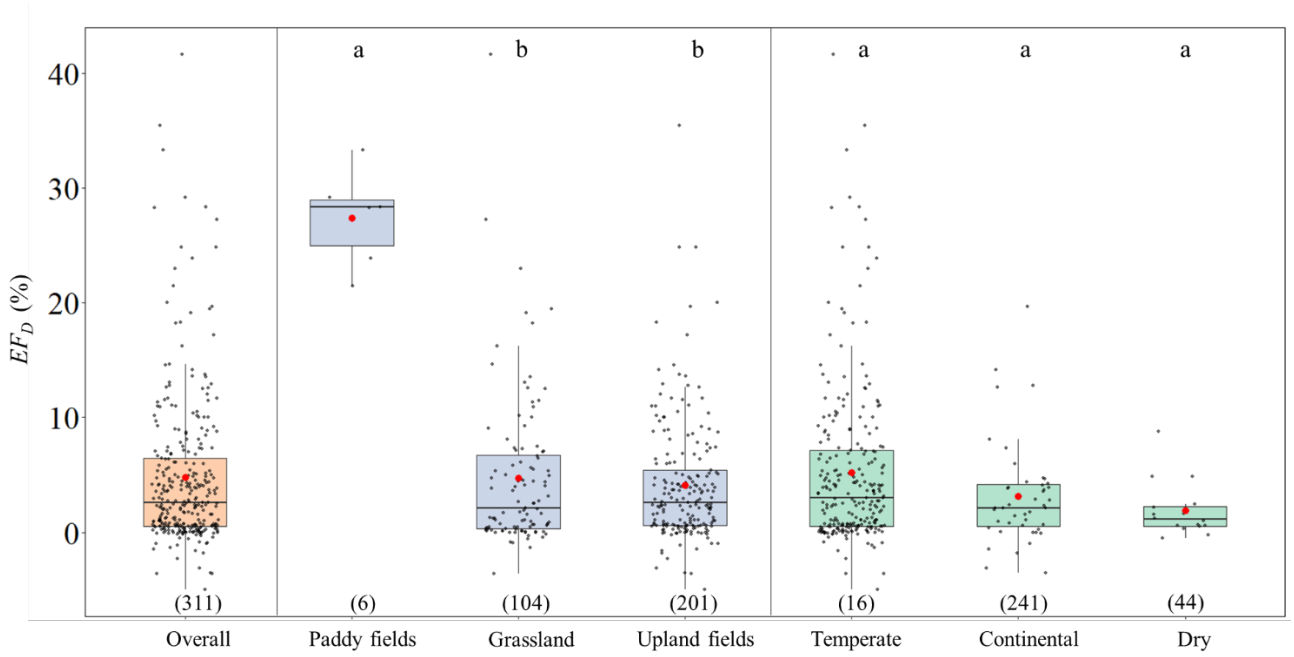

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597 Figure 3. EF_D across land uses and climatic zones from field experiments. Values in parentheses

598 represent the number of observations. Different letters on the top indicate that the mean values

599 are significantly different at $p < 0.05$.

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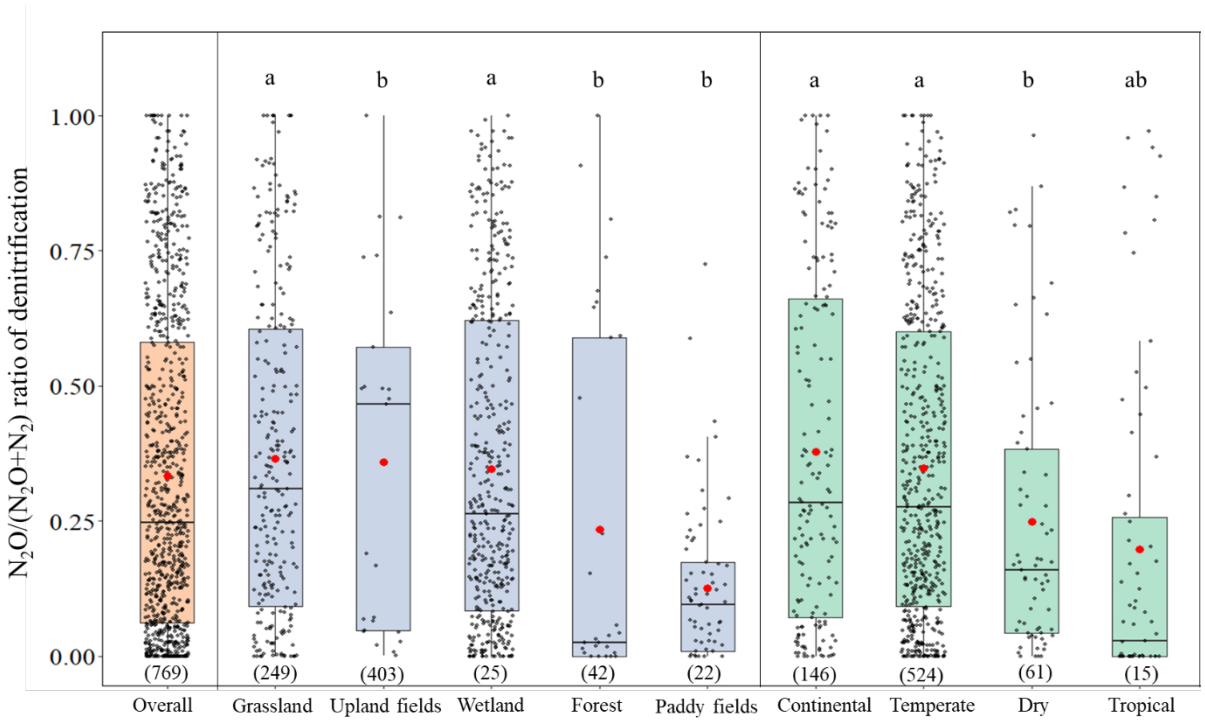
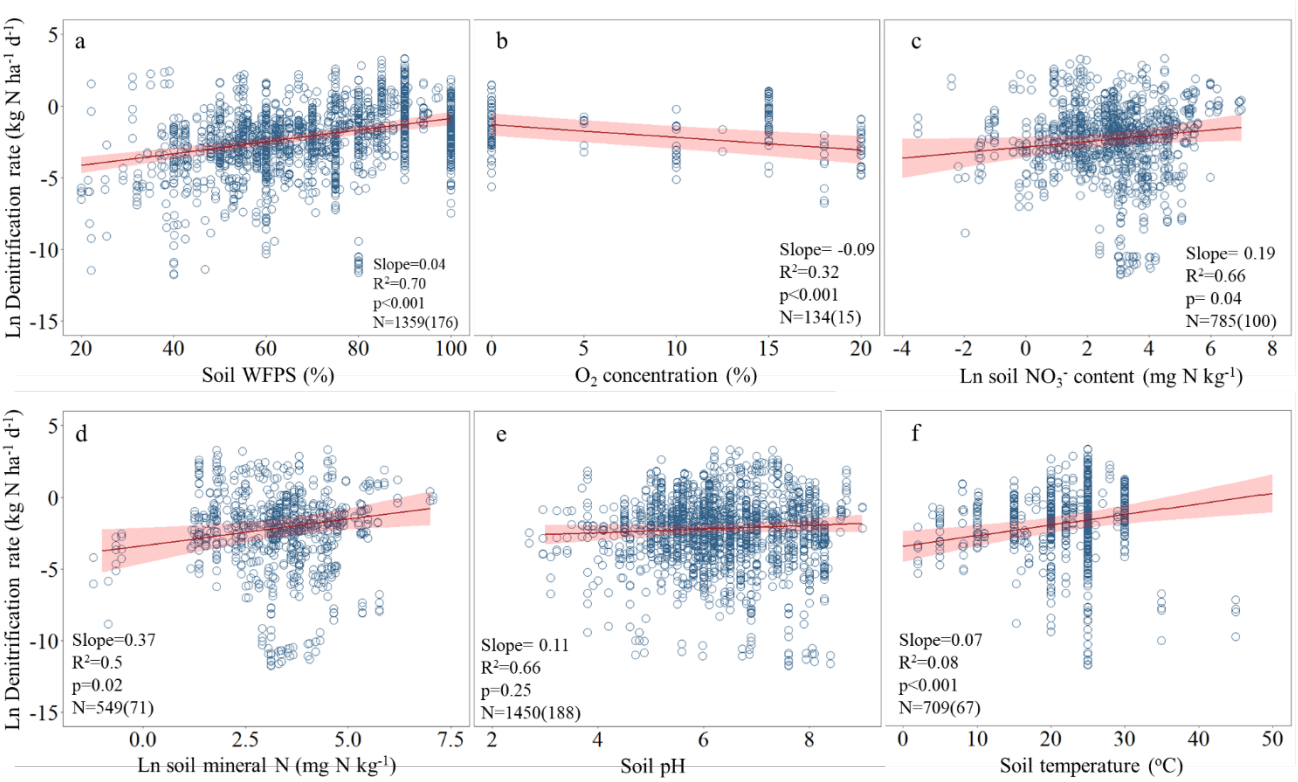


603

604 Figure 4. The $N_2O/(N_2O+N_2)$ ratio of denitrification across climatic zones and land use types.

605 Values in parentheses represent the number of observations. Different letters on the top indicate

606 that the mean values are significantly different at $p < 0.05$.



607

608 Figure 5. Relationships between soil denitrification rate and a) soil WFPS, b) soil O_2 concentration,
609 c) soil NO_3^- content, d) soil mineral N, e) soil pH and f) soil temperature. The red lines indicate
610 the slopes from the linear mixed effects models and the red shadings represent the 95% confidence
611 intervals. N in the figure represents the number of observations and the values in parentheses the
612 number of studies.

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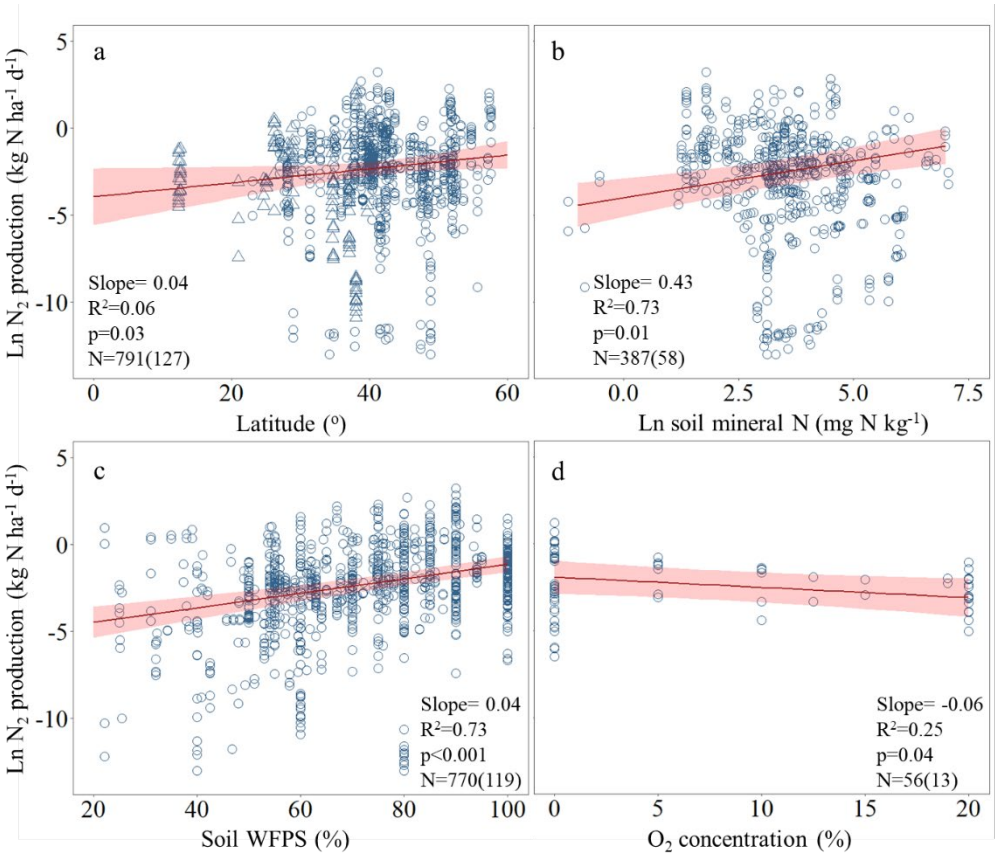

614

615 Figure 6. Relationships between soil N_2 emissions and a) latitude (circles: the Northern
616 Hemisphere; triangles: Southern Hemisphere), b) soil mineral N, c) soil WFPS and d) soil O_2
617 concentration. The red lines indicate the slopes from the linear mixed effects models and the red
618 shadings represent the 95% confidence intervals. N in the figure represents the number of
619 observations and the values in parentheses the number of studies.

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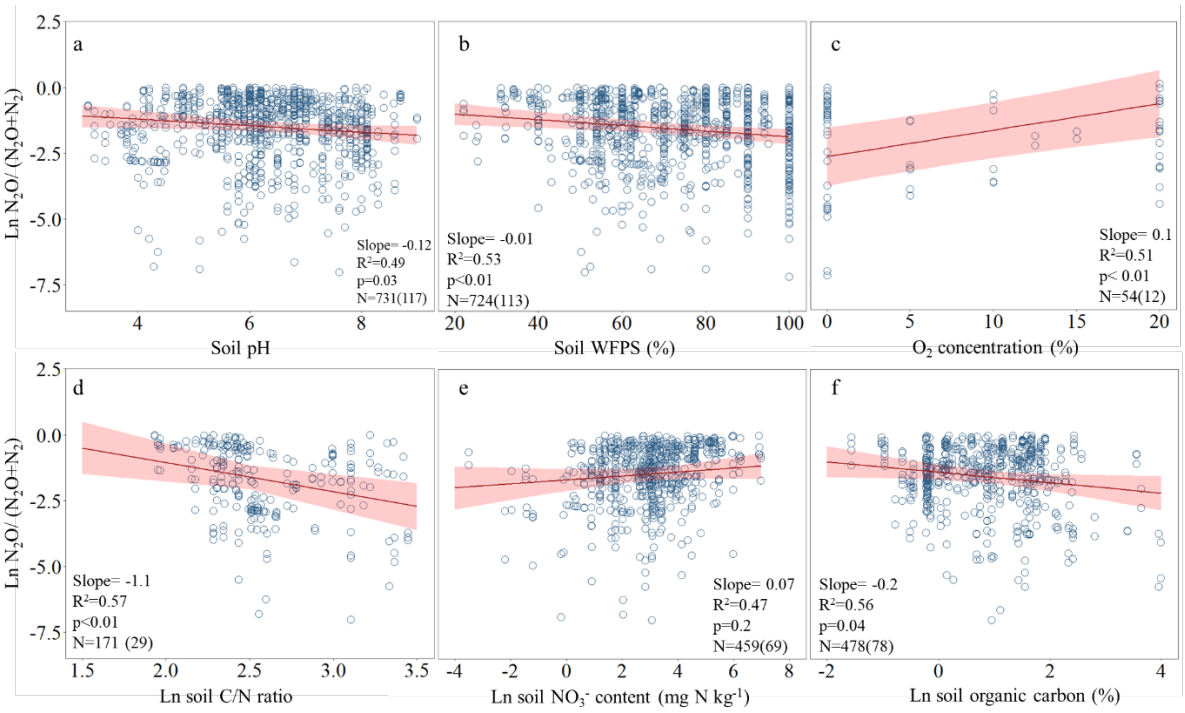

Figure 7. Relationships between soil $N_2O/(N_2O+N_2)$ ratio and a) soil pH, b) soil WFPS, c) soil O_2 concentration, d) soil C/N ratio, e) soil NO_3^- content and f) soil organic C. The red lines indicate the slopes from the linear mixed effects models and the red shadings represent the 95% confidence intervals. N in the figure represents the number of observations and the values in parentheses the number of studies.

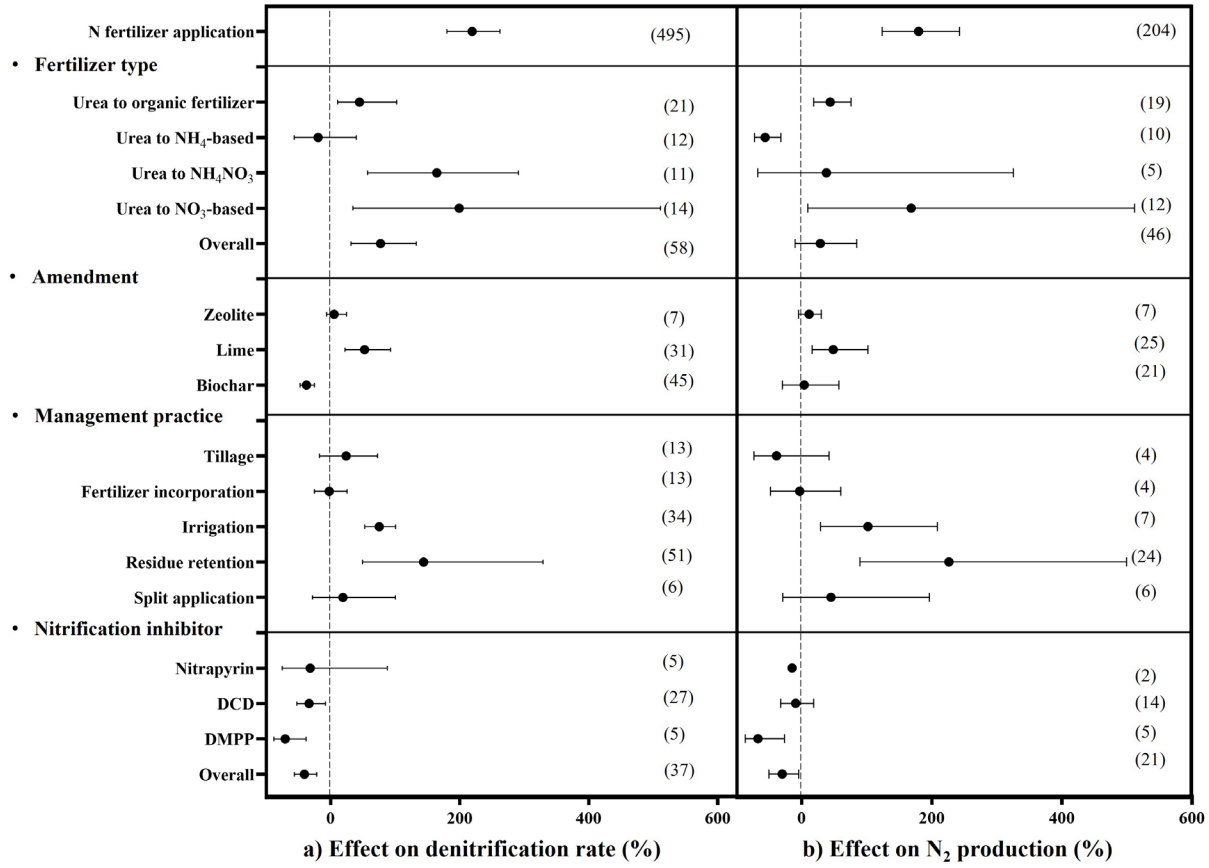

Figure 8. Effect of N application, fertilizer type, amendment, management practice and nitrification inhibitor on a) denitrification rate and b) N_2 production. Means and 95% confidence intervals are depicted. Numbers of experimental observations are in parentheses. DCD: dicyandiamide, DMPP: 3,4-Dimethylpyrazole phosphate, Nitrapyrin: 2-chloro-6-(trichloromethyl) pyridine.

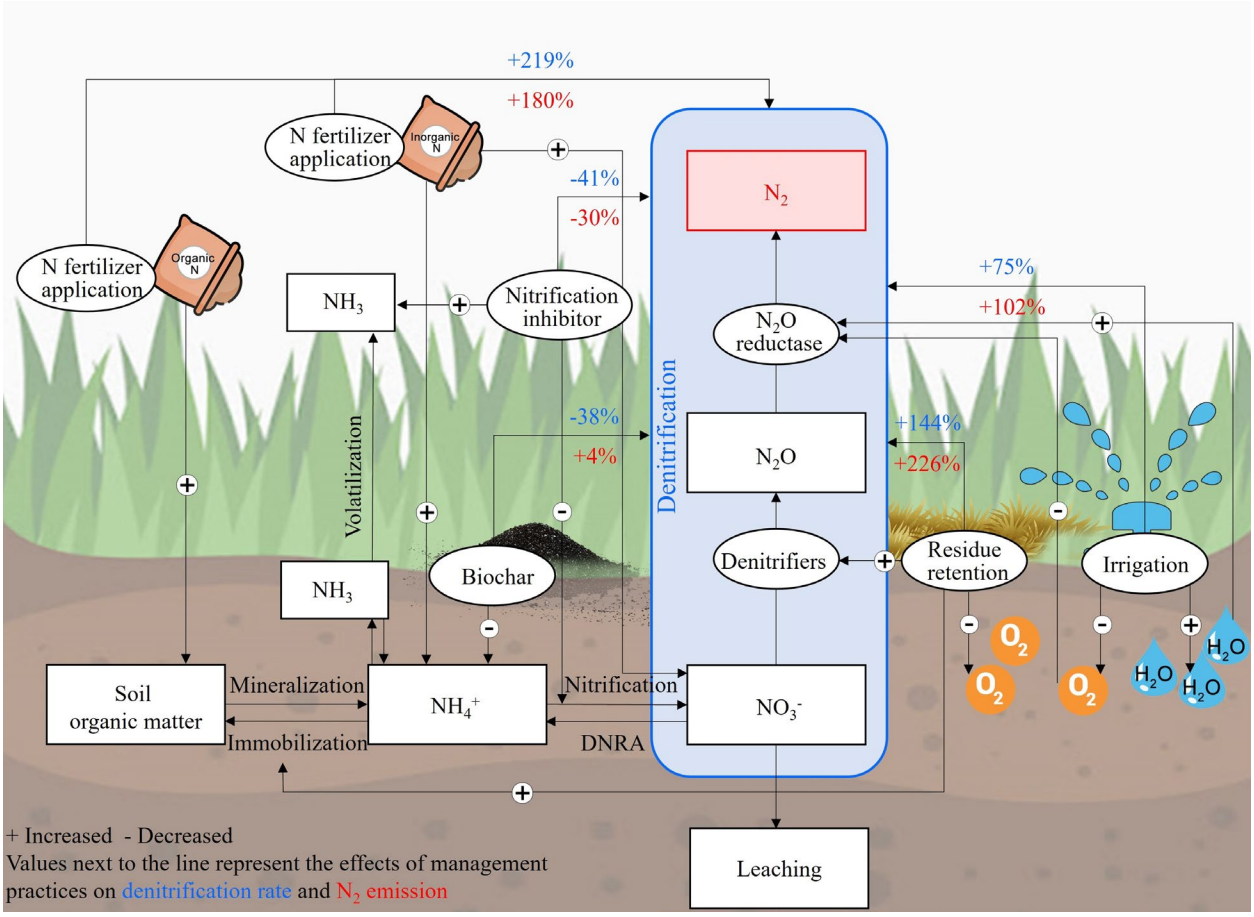

Figure 9. The effects of management practices on soil denitrification rate, N_2 emission and associated soil N transformations. Values presented were obtained from the meta-analysis in this study. '+' and '–'denote increased and decreased responses to management practices. DNRA: dissimilatory nitrate reduction to ammonium.