Denitrification at Sub-Zero Temperatures in Arable Soils: A Review

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Abstract Nitrogen (N) in agricultural fertilizers is denitrified by soil bacteria when oxygen is limited, which effectively removes plant-available N from the soil to the atmosphere. Reported denitrification rates range from 0 to 239 kg N ha^{-1} yr^{-1}, and, depending upon environmental conditions and management, may reduce the amount of N available for crop growth by 27%. Denitrification in soils also results in emissions of nitrous oxide (N_2O), which is a recognized pollutant that contributes to stratospheric ozone destruction and radiative forcing in the troposphere. Practitioners of sustainable agronomy aim to improve plant N-use efficiency and reduce emissions of the greenhouse gases by synchronizing N application and plant nutritional requirements. However, it is difficult to predict denitrification rates during and after the growing season based on current knowledge. High rates are consistently reported in irrigated cropping systems following heavy applications of fertilizer-N, but few studies report denitrification during the dormant season. Denitrification in winter may represent a significant sink for fertilizer-N in cropping systems, but further research at sub-zero soil temperatures is needed. Here, the three factors required for microbial denitrification: limited O_2 availability, electron donors and electron acceptors, are reviewed based on soil research performed both above and below 0°C. Gaps in the knowledge of denitrification rates in cropping systems, particularly when soils are frozen, are identified. Sustainable management of N in cropping systems such as greater N-use efficiency and lower greenhouse gas emissions could be advanced by greater understanding of denitrification in winter.

Keywords Fertilizer • Nitrogen • Nitrous oxide • Sub-zero temperatures

1 Introduction

The greatest agronomic uncertainty in balancing the nitrogen (N) budget of agricultural landscapes is the rate of denitrification, which converts plant-available N into gaseous N (Galloway et al. 2004). Specifically, it is not known when denitrification in the rooting zone reduces the availability of N to crops or the magnitude of N losses via denitrification. Current average N-use efficiency in cropping systems (% recovery of applied N) is reported to range from 30 to 50% (Cassman et al. 2002). A major reason for low N-use efficiency is the loss of gaseous-N from agricultural soils worldwide (Davidson and Seitzinger 2006). Denitrification may transfer up to 27% of agricultural N back to the atmosphere (Bouwman et al. 2005). However, spatial and temporal heterogeneity in denitrification rates, lack of quantitative data and inconsistencies between laboratory vs. field measurements contribute to uncertainties in the rate of denitrification, despite decades of research (Davidson and Seitzinger 2006).

Achieving synchrony between N supply and crop demand without sacrificing yield or protection of the environment requires greater knowledge of denitrification rates, yet knowledge of denitrification during the dormant season is limited. In many cases, gaseous-N

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losses at sub-zero soil temperatures are unknown or considered negligible. Consequently, wintertime N losses are rarely considered in crop fertilization recommendations. The body of evidence suggests microbes are physiologically active when soils are frozen (Clein and Schimel 1995; Mikan et al. 2002; Price and Sowers 2004; Rivkina et al. 2000), calling for agronomists to question what is known about the physicochemical and biological properties of soil below 0°C. Microbial emissions of N_2O and N_2 occur at sub-zero soil temperatures (Phillips 2007; Röver et al. 1998), but processes controlling denitrification rates in frozen soils are currently unknown. Gaps in the knowledge below 0°C need to be filled because there may be unforeseen opportunities for conservation of fertilizer-N and for reductions in greenhouse gas emissions during winter.

Denitrification is a ubiquitous process, occurring globally in both terrestrial and aquatic ecosystems (Davidson and Seitzinger 2006). This review focuses on denitrification through the process of anaerobic microbial respiration known to occur in sub-oxic soil microsites (Myrold and Tiedje 1985; Parkin 1987) specifically in cropped soils. Other, non-respiratory pathways observed in aquatic systems (e.g., chemodenitrification, aerobic ammonium oxidation) are outside the scope of this review (Hulth et al. 1999; Kuypers et al. 2005). Three fundamental factors are required for anaerobic microbial denitrification: (1) sub-oxic or anoxic conditions (herein referred to anoxic), (2) electron donors (herein referred to as organic C), and (3) electron acceptors nitrite (NO_2^-) or nitrate (NO_3^-), (herein referred to as NO_3^-). Each factor is reviewed separately with respect to denitrification when soil temperatures are greater or less than 0°C. Research that is necessary to unravel how denitrification might occur at sub-zero soil temperatures is proposed.

2 Denitrification Overview

Denitrification is classically defined as the microbial oxidation of organic matter, where NO_3^- is the terminal electron acceptor. It is a heterotrophic process of anaerobic respiration conducted by facultative bacteria using oxidized forms of N to accept electrons when O_2 is limited (Firestone et al. 1980). The end product is N_2, but some intermediate compounds (such as N_2O and NO) may also be produced, depending upon environmental conditions (Firestone and Davidson 1989). The primary factor controlling the rate of denitrification is O_2 availability because in sub-oxic conditions (<0.2 mg O_2 L^{-1}), some facultative microbes that normally use O_2 as an electron acceptor will use NO_3^- (Firestone et al. 1980; Seitzinger et al. 2006). Sub-oxic, as defined here, is three orders of magnitude lower than the density of O_2 in moist air (290 mg O_2 L^{-1}). Numerous laboratory incubation studies indicate that, for similar soils incubated at a constant temperature, denitrification rates can be manipulated by varying percent water-filled pore space (%WFPS), electron donors, and electron acceptors (Firestone and Davidson 1989; Myrold and Tiedje 1985; Sexstone et al. 1988). Numerous field studies indirectly point to these factors by reporting how differences in drainage (O_2 status), soil organic matter form and quantity (electron donors), and fertilization form and application (electron acceptors) alter rates of denitrification (Aulakh et al. 1984, 2001; Hofstra and Bouwman 2005). Accordingly, for a given soil and temperature, the kinetics of denitrification can largely be explained by these three factors.

An intermediate gaseous product of denitrification, N_2O, has received a great deal of attention (Bouwman et al. 1995; Davidson et al. 2000; Dobbie et al. 1999; Dobbie and Smith 2003; Jungkunst et al. 2006) because of its importance in the processes of ozone destruction and radiative forcing (Prather et al. 2001). The global warming potential of N_2O is nearly 300 times greater than CO_2 by mass (Forster et al. 2007). Two soil microbial processes, nitrification and denitrification typically produce this gas, so N_2O fluxes measured at the soil surface are not necessarily products of denitrification. Losses resulting from complete reduction of NO_3^- to N_2 are rarely measured because the large atmospheric background of N_2 makes it analytically difficult to detect small increases in N_2 from denitrification (Davidson and Seitzinger 2006).

While knowledge is growing with respect to cropping systems and surface fluxes of N_2O in winter (Kaiser et al. 1998; Maggiotto and Wagner-Riddle 2001; Wagner-Riddle et al. 1997), measurements of total gaseous losses of N via denitrification in frozen soils are lacking. Observed fluxes of N_2O at the surface of frozen soils suggest microbial denitrification may occur at sub-zero soil temperatures (Röver et al. 1998) but specific mechanisms are unknown. One question is: how much total N (N_2O + N_2) is from

denitrification below 0°C and how much is degassed from accumulated products of denitrification prior to freezing?

Current knowledge of how management influences denitrification rates is largely garnered from experiments conducted during the growing season and at laboratory temperatures. Many field and laboratory experiments are conducted by manipulating the three factors required for denitrification (O_2 status, organic C, NO_3^-). For example, greater denitrification rates are found in fertilized cropped soils at high %WFPS (Barton et al. 1999; Hofstra and Bouwman 2005) because O_2 diffusion is restricted under saturated conditions and the proportion of soil volume that is anoxic increases (Sexstone et al. 1985; Smith 1980). In some cases, organic C and N additions will increase denitrification rates (Burford and Bremner 1975; Myrold and Tiedje 1985; Paul et al. 1993). Fertilizer-N and residue inputs might fuel denitrification in frozen soils at high moisture levels during the winter, but empirical studies are needed to estimate N transformation rates and controls in frozen soil. Filling these knowledge gaps during the winter will improve agronomic recommendations, with potentially positive economic and environmental benefits.

3 Water-Filled Pore Space in Frozen Soil

Application of soil %WFPS (1) as a proxy for soil O_2 status is fundamental to current models of denitrification (Firestone and Davidson 1989).

$$\%\text{WFPS} = (\theta_v/\phi) \times 100 \quad (1)$$

where

θ_v = percent volumetric water content, $\theta_m \times \rho_b$

ϕ = percent total porosity = $(1 - \rho_b/\rho_p) \times 100$

θ_m = percent gravimetric water content

ρ_b = soil bulk density (mg m^{-3})

ρ_p = soil particle density (~2.65 mg m^{-3}).

It is a well accepted approximation that at soil %WFPS >70 (where water is liquid), gaseous N emissions are the result of microbial denitrification (Bateman and Baggs 2005; Davidson 1991), although exact %WFPS values vary with soil mineralogy. It is less clear how %WFPS influences microbial denitrification when soil water is transformed to ice. Effects of freezing on the soil physical environment may influence rates of microbial denitrification at sub-zero soil temperatures.

Calculation of soil %WFPS becomes less tractable at sub-zero soil temperatures because the majority of liquid water becomes ice, rendering changes in bulk density (Kay et al. 1985), hydraulic conductivity (Pikul and Allmaras 1985), pore space volume (Loch and Kay 1978), and water content (Pikul et al. 1989). Liquid water in frozen soil is mobile (Pikul and Allmaras 1985) and flows along unfrozen liquid water channels (Edwards and Cresser 1992), which change in thickness as temperature decreases (Anderson and Hoeckstra 1965). Formation of ice pushes soil particles apart to increase soil pore size (Loch and Miller 1975), and ice lenses forms to create additional pores (Kay et al. 1985). The percent of water occupying soil pores is not constant because liquid water content and soil pore space are not constant at sub-zero soil temperatures.

The presence of both ice and unfrozen water in soil could enhance denitrification at oxic/sub-oxic interfaces controlled by the thermal gradient. Oxic/sub-oxic interfaces facilitate transport of oxidized forms of N from oxic to sub-oxic zones (Seitzinger et al. 2006). If these interfaces are present in frozen soil, then the amount of ice vs. unfrozen water could influence denitrification rates. However, the presence of oxic/sub-oxic interfaces is not likely to remain static in frozen soil because the amount of unfrozen water, the thickness of the water films, the size of transport channels, and hydraulic conductivity are controlled by soil temperature (Hoeckstra 1966; Pikul and Allmaras 1985). The temperature gradient continuously transforms ice to films of water (Kay et al. 1985), potentially creating sites for denitrification at oxic/sub-oxic interfaces.

Freezing also induces changes in soil structural stability (Bullock et al. 1988; Lehrsch et al. 1990), which interacts with water and temperature to affect soil pore space. Liquid water is replaced by ice lenses that weaken soil aggregates (Bullock et al. 1988; Edwards and Cresser 1992; Lehrsch et al. 1990). As the frost front moves into the soil and the majority of soil water is transformed to ice, soil cohesion is lost to shearing forces (Bullock et al. 1988). Slightly soluble chemicals precipitate at the surface of soil particles. As the thermal gradient vacillates diurnally and seasonally, ice crystals collapse and return to unfrozen water. As freezing progresses deeper into the soil, water also

migrates upward towards the freezing front to increase water content near the surface (Hoeckstra 1966). Soil aggregates frozen at high water contents (>15% v/v) will be more strongly affected by freezing than soil aggregates frozen at lower water contents (Lehrsch et al. 1990), with greater loss of aggregate stability and cohesion across a wide range of soil series (Bullock et al. 1988).

Disruption to the soil matrix as a result of freezing, as described above, is complex and dynamic. Freezing affects soil volume, migration of water, aggregate stability, precipitation of solutes, ice crystal formation, and ice crystal collapse – all of which could alter soil O_2 status. Microbes can remain physiologically active when films of unfrozen soil water are present (Mikan et al. 2002; Priemé and Christensen 2001; Rivkina et al. 2000), and denitrification has been measured in soils at –2 °C (Dorland and Beauchamp 1991; Phillips 2007). Initial %WFPS at freezing may be fundamental to understanding interactions between soil physics and soil microbial activity, but diffusion of O_2 may be limited by other factors. Current %WFPS thresholds for denitrification (Firestone and Davidson 1989) in frozen soil may need adjustment and/or other potential factors (e.g., thermal gradient, ice-filled pore space) considered.

4 Soil Organic Carbon in Frozen Soil

Organic C often limits denitrification in cropped soils at soil temperatures above 0°C (Beauchamp et al. 1989; Burford and Bremner 1975; McCarty and Bremner 1993; Sainz Rozas et al. 2001), but it is uncertain how organic C influences denitrification below 0°C. Alternatively, studies of aerobic microbial respiration below 0°C suggest soil organic C can limit microbial activity (Feng et al. 2007; Michaelson and Ping 2003; Priemé and Christensen 2001; Schimel and Clein 1996) and that aggregate disruption from freezing releases potentially mineralizable C to microbes when soils thaw (Christensen and Tiedje 1990; Priemé and Christensen 2001). Greater microbial respiration observed following freeze-thaw cycles (Christensen and Tiedje 1990; Mikan 2002; Teepe et al. 2004) may be linked to soil organic C (Breitenbeck and Bremmer 1986; Mikan 2002; Schimel and Clein 1996; Skogland et al. 1988), since freeze-thaw events positively influence amounts of small, hydrophilic compounds (Michaelson and Ping 2003) and phospholipid fatty acids (Feng et al. 2007). Suggested mechanisms for C availability following freezing and thawing include rupture of cellular membranes in microbial biomass (Skogland et al. 1988), the release of organic matter previously bound in aggregates (Christensen and Christensen 1991), and exposure of fresh reactive surfaces (Edwards and Cresser 1992). In addition, thawing may enhance the availability of C required for anaerobic respiration through the collapse of ice crystals and diffusion of substrate to anoxic microsites.

Rarely are effects of freezing on microbial activity separated into components of C availability vs. physical soil disturbance. Reported pulses of aerobic microbial respiration in frozen soils may be due to disturbance from aggregate disruption (Edwards and Cresser 1992), release of organic C (Christensen and Christensen 1991; Myrold and Tiedje 1985; Schimel and Clein 1996), changes in the form of organic C (Feng et al. 2007), or to changes in the diffusion of gases or solutes in frozen soil. The causative factor of respiratory activities (physical disturbance, substrate amount/form, diffusion) likely contributes to the magnitude and duration of observed pulses. This may be critical to denitrification questions in agronomy because physical disturbances from freezing can be modified, to some extent, with tillage and residue management. Diurnal and seasonal soil temperature extremes are modulated and depth to frost is reduced with standing stubble (Pikul et al. 1986), with mulch application (Kohnke and Werkhoven 1963), and with reduced tillage (Kay et al. 1985). On the other hand, carbon can limit denitrification (McCarty and Bremner 1993), and addition of plant residues can promote denitrification activity (Aulakh et al. 1984; McCarty and Bremner 1993). Laboratory studies using soils from no-till cropping systems point to greater soil C as the reason for higher denitrification rates, compared to conventional tillage (Aulakh et al. 1984; Liu et al. 2007; van Bochove et al. 2000). Understanding and quantifying effects of management on denitrification in frozen soil require separation of physical disturbance (loss of aggregate cohesion and stability) from release and transport of organic C potentially bound in soil aggregates. From here, if organic C is

limiting denitrification, both tillage and residue management recommendations could be balanced to potentially reduce denitrification rates.

5 Nitrogen in Frozen Soils

Effects of fertilizer-N on denitrification have been studied extensively (see review by Hofstra and Bouwman 2005). Fertilized soil at high %WFPS will promote facultative anaerobic bacteria to reduce N oxides and consume organic matter (Firestone and Davidson 1989; Mulvaney et al. 1997). The magnitude and duration of NO_3^- additions varies with soil texture, pH, climate, crop, management, etc (Jungkunst et al. 2006; Kaiser et al. 1998; Nieder et al. 1989; Sainz Rozas et al. 2001). It is generally accepted that cropped soils with high rates of fertilizer-N inputs generally exhibit higher denitrification rates than soils not receiving fertilizer-N additions (Aulakh et al. 2001; Barton et al. 1999; Jarvis et al. 1991; Kaiser et al. 1998). Manures and animal slurries amendments also enhance denitrification rates (Calderón et al. 2004; Ginting et al. 2003; Lessard et al. 1996; Mogge et al. 1999; Paul et al. 1993; Petersen 1999). The proportion of fertilizer-N denitrified in crop fields varies widely across soil series and climates (see review by Nieder et al. 1989), with 2.5% reported in Colorado, USA (Mosier et al. 1986) and 60% reported in Denworth, UK (Colbourn et al. 1984).

The effect of fertilizer-N application on denitrification rates in cropped soils during the winter is less known, particularly when soils are frozen. Field studies instead have reported significant emissions of N_2O (Goossens et al. 2001; Maggiotto and Wagner-Riddle 2001; Ruser et al. 2001; Wagner-Riddle et al. 1997). Nitrous oxide studies are labor intensive, and microbial activity in frozen soil is often assumed to be negligible; consequently N_2O flux data collected at the surface of frozen soil are rare (Phillips 2007; Röver et al. 1998). Evidence of greater microbial N_2O production in fertilized soil during winter suggests that denitrification may occur in anoxic soil microsites at low soil temperatures. Manure amendments were found to increase N_2O flux and denitrification in the field and in soil cores (30 cm depth) incubated at sub-zero soil temperatures (Phillips 2007), but further research is needed to determine the geographic prevalence of denitrification in frozen soils, and how form and timing of fertilizer-N application might alter gaseous N losses in winter.

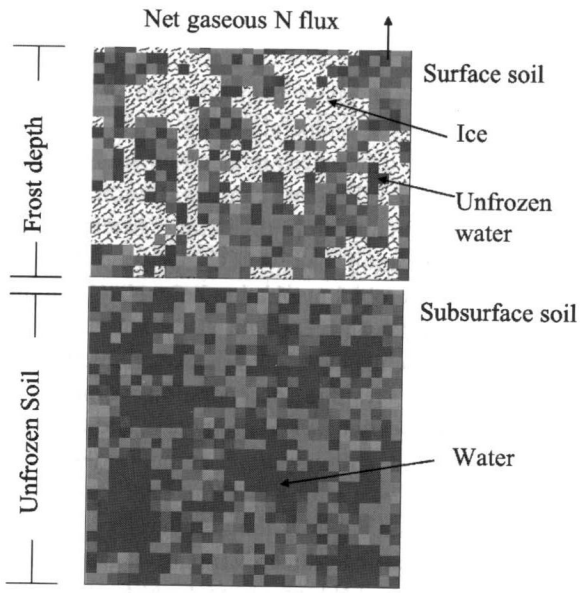

Fig. 1 Conceptual illustration of a soil column where ice and unfrozen liquid water exist in surface soil (frozen soil depth varies with frost depth) and liquid water exists in subsurface soil. *Brown* represents soil, *blue* represents water, and *white with blue* represents ice

Determining the location of denitrification in the soil profile is also paramount to understanding N management in winter (Fig. 1). Denitrification in the soil profile could occur (1) below the freezing front in subsurface soil, (2) in the frozen surface soil, (3) at the interface between frozen/unfrozen soils, or (4) throughout the profile in both frozen and unfrozen soil. In each case, the distance denitrification products would need to travel upward to the surface would significantly affect emissions at the surface because there may be opportunities for complete reduction of NO or N_2O to N_2 as they migrate from below the freezing front to the surface. Further, as suggested earlier, denitrification controls in the frozen surface soil are likely different from denitrification controls in the unfrozen subsurface. Snow cover, residue cover, and tillage will reduce the frozen soil depth; consequently, understanding the vertical distribution of denitrification activity within the soil profile will point to how management might influence N emissions at the soil surface by manipulating soil temperature.

6 Conclusion

What is not known about denitrification in cropped soils at sub-zero soil temperatures far exceeds what is known. Physical differences induced by soil freezing suggest use of %WFPS as a proxy for soil aerobic status is insufficient for predicting heterotrophic anaerobic respiration below 0°C. Organic C may limit aerobic microbial respiration below 0°C, calling into question if organic C might also limit anaerobic respiration. If so, management practices implemented in autumn (e.g., residue incorporation, compost or fertilizer-N application) could promote N and C losses via denitrification, with important agronomic implications (Fig. 2). Moreover, observed fluxes of N_2O in fertilized cropped soils during the dormant season point to potential losses of fertilizer-N inputs via denitrification, but total annual N losses need quantification. The agronomic importance of timing and form of fertilizer-N during the growing season is well known. Less known is how post-season N application influences the N-budget and plant-available N the following spring. Denitrified N losses from cropping systems at sub-zero soil temperatures may or may not amount to a significant portion of the N budget. However, the preponderance of the evidence suggests denitrification should not be considered negligible without further investigation.

A number of agronomic research questions have been raised with respect to the three factors required for denitrification (limited O_2, organic C, NO_3^-), a few of which are summarized below.

- How much N is denitrified during the off-season, particularly when soils are frozen?
- How does water content below 0°C influence denitrification compared to above 0°C?
- What is the O_2 status of frozen soil and how does this change with the advancement of the freezing front?
- How does migration of water below 0°C influence denitrification?
- Is denitrification in frozen soil limited by organic C? How available is organic C to microbes below 0°C?
- How is substrate transport affected by soil comprised of ice and unfrozen soil water? How available are solutes to microbes in frozen soil?
- How is fertilizer-N transformed in frozen soil? At what point in the N-cycle is N transformation inhibited by freezing temperatures?
- Does the type of fertilizer-N applied to crop fields (e.g., compost, urea, anhydrous ammonium) influence denitrification in winter?
- Do plant residue fermentation products enhance denitrification below 0°C?
- Does denitrification occur in both the frozen soil near the surface and in the unfrozen subsurface soil?

The evidence indicates microbial denitrification occurs during the winter in previously-cropped soils at sub-zero soil temperatures, and the potential exists for some mediation with management. However, management studies should be preceded by basic knowledge of how frozen soil conditions alter soil O_2 status and anaerobic transformation of NO_3^- to gaseous N_2O and N_2. From there, questions of soil pH, texture, residue quantity and quality, fertilizer-N form and timing, etc., can be more parsimoniously addressed. Potential economic and climate change implications warrant continued, mechanistic research.

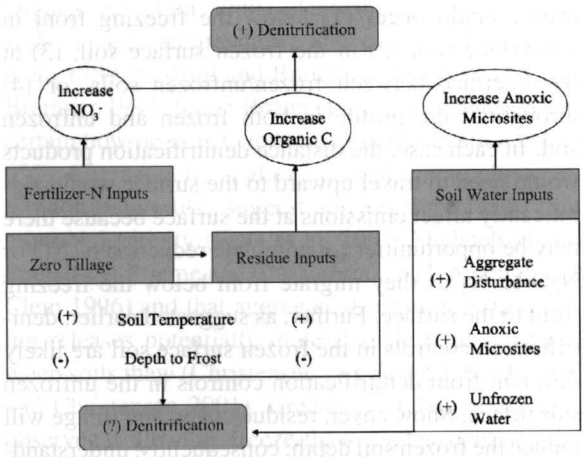

Fig. 2 Summary of potential crop management effects on denitrification in frozen soil. Positive effects are indicated with (+) and negative effects by (−). Some of the indirect effects of management on denitrification in soil, designated by (?), are unknown

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