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# Denitrification in cropping systems at sub-zero soil temperatures. 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<sup>-1</sup> yr<sup>-1</sup>, 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<sub>2</sub>O), 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<sub>2</sub> 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.

**nitrous oxide / sub-zero temperatures / nitrogen / fertilizer**

## 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 30–50% (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 versus 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 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, 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<sub>2</sub>O and N<sub>2</sub> 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 as anoxic), (2) electron donors (herein referred to as organic C), and (3) electron acceptors nitrite (NO<sub>2</sub><sup>-</sup>) or nitrate (NO<sub>3</sub><sup>-</sup>), (herein referred to as NO<sub>3</sub><sup>-</sup>). Each factor is reviewed

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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  $\text{NO}_3^-$  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  $\text{O}_2$  is limited (Firestone et al., 1980). The end product is  $\text{N}_2$ , but some intermediate compounds (such as  $\text{N}_2\text{O}$  and  $\text{NO}$ ) may also be produced, depending upon environmental conditions (Firestone and Davidson, 1989). The primary factor controlling the rate of denitrification is  $\text{O}_2$  availability because in sub-oxic conditions ( $<0.2 \text{ mg O}_2 \text{ L}^{-1}$ ), some facultative microbes that normally use  $\text{O}_2$  as an electron acceptor will use  $\text{NO}_3^-$  (Firestone et al., 1980; Seitzinger et al., 2006). Sub-oxic, as defined here, is three orders of magnitude lower than the density of  $\text{O}_2$  in moist air ( $290 \text{ mg O}_2 \text{ L}^{-1}$ ). 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; Sexton et al., 1988). Numerous field studies indirectly point to these factors by reporting how differences in drainage ( $\text{O}_2$  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,  $\text{N}_2\text{O}$ , 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  $\text{N}_2\text{O}$  is nearly 300 times greater than  $\text{CO}_2$  by mass (Forster et al., 2007). Two soil microbial processes, nitrification and denitrification typically produce this gas, so  $\text{N}_2\text{O}$  fluxes measured at the soil surface are not necessarily products of denitrification. Losses resulting from complete reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  are rarely measured because the large atmospheric background of  $\text{N}_2$  makes it analytically difficult to detect small increases in  $\text{N}_2$  from denitrification (Davidson and Seitzinger, 2006).

While knowledge is growing with respect to cropping systems and surface fluxes of  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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 ( $\text{N}_2\text{O} + \text{N}_2$ ) 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 ( $\text{O}_2$  status, organic C,  $\text{NO}_3^-$ ). For example, greater denitrification rates are found in fertilized cropped soils at high %WFPS (Barton et al., 1999; Hofstra and Bouwman, 2005) because  $\text{O}_2$  diffusion is restricted under saturated conditions and the proportion of soil volume that is anoxic increases (Sexton 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 (Eq. (1)) as a proxy for soil  $\text{O}_2$  status is fundamental to current models of denitrification (Firestone and Davidson, 1989).

$$\%WFPS = (\theta_v / \phi) \times 100 \quad [1]$$

where  $\theta_v$  = percent volumetric water content,  $\theta_m \times \rho_b$ ;

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

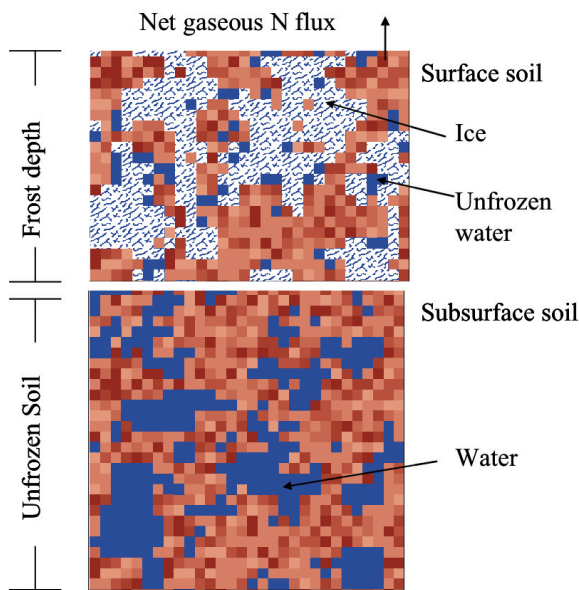
$\theta_m$  = percent gravimetric water content;

$\rho_b$  = soil bulk density ( $\text{mg m}^{-3}$ );

$\rho_p$  = soil particle density ( $\sim 2.65 \text{ mg m}^{-3}$ ).

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 Hoekstra, 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.



**Figure 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.

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 versus 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 (Hoekstra, 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; Lehrs 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; Lehrs 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 (Hoekstra, 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 (Lehrs 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<sub>2</sub> 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<sub>2</sub> 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 Klein, 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; Jacinthe et al., 2002; Mikan, 2002; Teepe et al., 2004) may be linked to soil organic C (Breitenbeck and Bremner, 1986; Mikan, 2002; Schimel and Klein, 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 versus 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 Klein, 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  $\text{NO}_3^-$  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  $\text{N}_2\text{O}$  (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  $\text{N}_2\text{O}$  flux data collected at the surface of frozen soil are rare (Phillips, 2007; Röver et al., 1998). Evidence of greater microbial  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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.

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 (a) below the freezing front in subsurface soil, (b) in the frozen surface soil, (c) at the interface between frozen/unfrozen soils, or (d) 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  $\text{NO}$  or  $\text{N}_2\text{O}$  to  $\text{N}_2$  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.

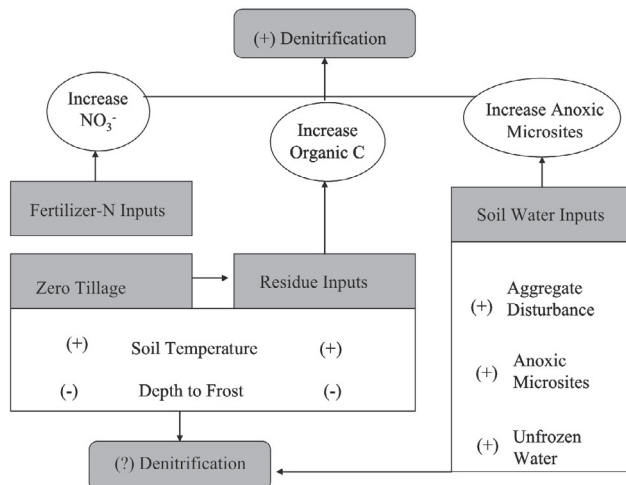
## 6. CONCLUSION

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  $\text{N}_2\text{O}$  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  $\text{O}_2$ , organic C,  $\text{NO}_3^-$ ), a few of which are summarized below.

- How much N is denitrified during the off-season, particularly when soils are frozen?

## Crop Management Practices and Potential Effects on Denitrification in Frozen Soil



**Figure 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, denominated by (?), are unknown.

- How does water content below 0 °C influence denitrification compared to above 0 °C?
- What is the O<sub>2</sub> 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<sub>2</sub> status and anaerobic transformation of NO<sub>3</sub><sup>-</sup> to gaseous N<sub>2</sub>O and N<sub>2</sub>. 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.

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