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## Gross N-cycling Rates in Ephemeral Wetlands

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**Key Words:** mineralization, nitrification, N turnover,  $^{15}\text{N}$  isotope dilution

### Abstract

Ephemeral wetlands or depressions in hummocky landscapes have high levels of C, N, and soil moisture, often leading to high nutrient cycling activity. However, measuring soil nitrate and ammonium pools is typically a poor indication of N-cycling activity or of the soil N that is available for other processes such as  $\text{N}_2\text{O}$  emissions. This study used stable  $^{15}\text{N}$  isotope dilution techniques in cultivated and uncultivated ephemeral wetlands in central Saskatchewan to quantify land use effects on gross mineralization and nitrification rates. In-field incubation experiments were repeated in early May, mid-June and late July. There was a clear land use effect on inorganic soil N levels, with significantly less  $\text{NH}_4^+$  and more  $\text{NO}_3^-$  in the cultivated wetland soils. However, the rates of  $\text{NH}_4^+$  mineralization and  $\text{NO}_3^-$  nitrification were similar for both land uses, indicating similar substrate availability but different N-consuming processes. Both N pools turned over in as little as 1-2 d, highlighting the ineffectuality of measuring inorganic N pools as a predictor for N availability in these soils.

### Introduction

Hummocky till landscapes are the dominant geomorphic surface in the Prairie Pothole region, which extends from East-Central Alberta through to North-Central Iowa. These landscapes contain a very high number of wetlands – for example, our research site near St. Denis, Saskatchewan, Canada contains 216 wetlands in an area of  $3.84 \text{ km}^2$  (Hogan and Conly, 2002) – interspersed within the cultivated upland portions of the landscape. Many of the wetlands have been cleared of natural vegetation and are now used for annual crop production. This conversion from native wetland to cultivated wetlands has presumably altered the rates of fundamental N processes but to date little or no information is available on the rates of N-cycling processes in these landscape positions.

Cropped landscapes receive nitrogen (N) inputs in the form of inorganic and/or organic fertilizers, and these inputs have a significant impact on local and regional N cycling, N gas losses, and N leaching (Burke et al., 2002). N-cycling processes of particular interest for both agricultural and environmental concerns are: 1) mineralization, which produces  $\text{NH}_4^+$  from the soil organic N pool and 2) nitrification, which produces  $\text{NO}_3^-$  from the  $\text{NH}_4^+$  and organic N pools and emits nitrous oxide ( $\text{N}_2\text{O}$ ) as a by-product. Nitrous oxide is a greenhouse gas with 296 times the global warming potential of  $\text{CO}_2$ . In Canada, agriculture accounts for up to 70% of anthropogenic  $\text{N}_2\text{O}$  emissions (Janzen et al., 1998).

Typical assessments of N pool status and activity include “snapshot” tests of soil  $\text{NH}_4^+$  or  $\text{NO}_3^-$  pools at one or more times in the growing season, or measures of net mineralization and nitrification (i.e., the net increase or decrease in  $\text{NH}_4^+$  or  $\text{NO}_3^-$  over a given period of time). However, researchers are increasingly recognizing that these measures provide limited information about N-cycling activity in the soil and are turning to stable isotope dilution techniques to measure gross rates of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  production (Booth et al., 2005; Davidson et al., 1991; Hart et al., 1994). In US ecosystems with more temperate climates than the Prairie Pothole region, lower slopes have been shown to have higher rates of both gross  $\text{NH}_4^+$  mineralization and immobilization (Verchot et al., 2002) and lower rates of gross nitrification compared to upper slopes (Corre et al., 2002), emphasizing the significance of landform position in N-cycling processes. Research in the Pothole region of Saskatchewan has shown that lower slopes have higher SOC stores and lower  $\text{N}_2\text{O}$  emissions in uncultivated soils compared to cultivated ones, emphasizing the overlying effects of land use on landform patterns (Corre et al., 1999; Pennock, 2003).

Examining N-cycling processes in the context of land use will improve our ability to manage agricultural landscapes to minimize N losses, advancing sustainable agriculture while addressing concerns surrounding greenhouse gas emissions and global change. This study used stable  $^{15}\text{N}$  isotope techniques in cultivated and uncultivated ephemeral wetlands in central Saskatchewan to quantify gross mineralization and nitrification by land use. These measurements will be compared with the more ubiquitous measurements of soil inorganic N levels.

## Methods

This project was carried out in the St. Denis National Wildlife Area (SDNWA) (Latitude:  $52^\circ 12'$ , Longitude:  $106^\circ 5'$ ) of central Saskatchewan, Canada. The SDNWA is in the Dark Brown soil climatic zone and is dominated by hummocky terrain with loamy unsorted glacial till (Weyburn association) parent materials; slope classes range from 10 to 15% (Miller et al., 1985). Within the SDNWA, five ephemeral wetlands were selected: 3 uncultivated wetlands (2 were cultivated prior to 1968) and 2 cultivated wetlands. Ephemeral wetlands are those depressions in hummocky landscapes that contain standing water in the spring, but typically dry up during the growing season (Hayashi et al., 1998). The cultivated wetlands were direct-seeded to wheat on May 8, 2005. Granular fertilizer containing 72% 46-0-0 and 28% 11-52-0 was placed with seed at a rate of  $135 \text{ kg ha}^{-1}$ .

Each of the 5 wetlands was sampled three times throughout the 2005 growing season: May 4 (post-snowmelt), June 16 (early growing season), and July 26 (mid-growing season). Within each uncultivated wetland, sampling was focused in the riparian or willow ring soils, not the pond centers. For the cultivated wetlands, sampling was from the analogous concave footslope elements (Pennock et al., 1987). Three sub-sampling points were selected for each wetland ( $5 \times 3 = 15$  points in total). On a given sampling date, five intact cores were taken per sub-sampling point: one for bulk density and gravimetric soil moisture measurements and four for the isotope dilution field incubation experiment. Cores (5-cm I.D.) were taken to a 15-cm depth, corresponding to the average thickness of the A horizon, using a slide hammer sampler equipped with a removable plastic liner (AMS Signature Series).

Of the four isotope-dilution cores, one pair was labeled with  $(^{15}\text{NH}_4)_2\text{SO}_4$  (for gross  $\text{NH}_4$  mineralization) and the other pair was labeled with  $\text{K}^{15}\text{NO}_3$  (for gross nitrification); each solution contained  $30 \mu\text{g N ml}^{-1}$  at 98%  $^{15}\text{N}$  enrichment. Labeling was done using seven 2-ml injections per core with an 18-gauge side-port spinal needle after Davidson et al. (1991). Solution injections increased the gravimetric soil moisture by  $7\pm 2\%$ . With the exception of those uncultivated cores with the lowest initial concentrations of soil  $\text{NO}_3^-$  (where  $\text{NO}_3^-$  injections as much as doubled the soil  $\text{NO}_3^-$  levels) adding inorganic N did not significantly increase the soil N levels relative to the inherent background variability observed within each wetland.

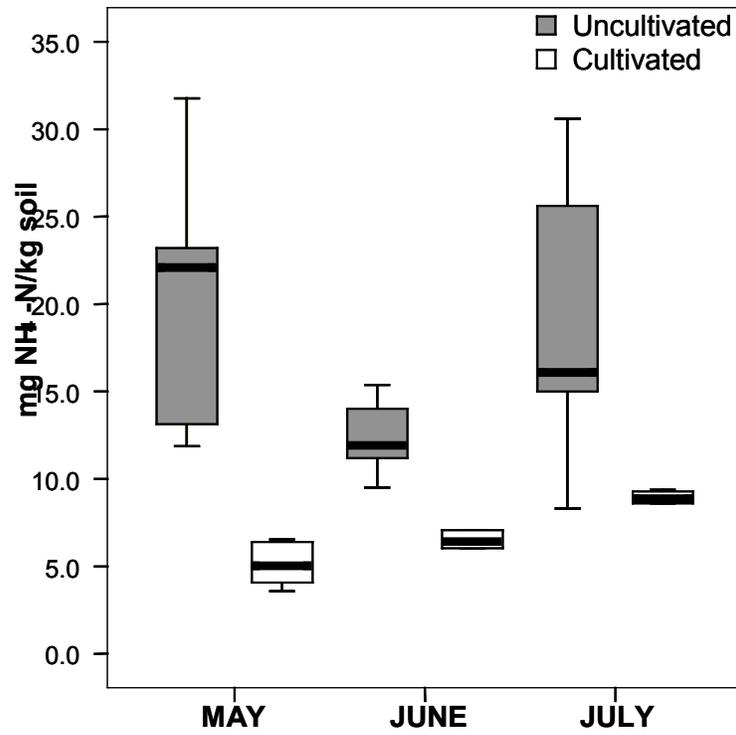
One core of each pair was broken up within 1 h of injection ( $t=0$ ), sub-sampled and extracted using 2M KCl (on site). The other core was capped and buried on site for 24 h before extraction. The KCl extracts were analyzed for inorganic N concentration colorimetrically using a Technicon AutoAnalyzer (Technicon Industrial Systems, 1978) and analyzed for  $^{15}\text{NH}_4$  and  $^{15}\text{NO}_3$  using the modified diffusion procedure outlined in Bedard-Haughn et al. (2004). The differences in N concentration and  $^{15}\text{N}$  content between the  $t=0$  and  $t=24$  h samples were used to calculate gross mineralization and nitrification according to Hart et al. (1994). Turnover rate, the rate at which a given inorganic N pool replaces itself, was calculated as the  $\text{NH}_4^+$  pool size divided by the gross mineralization rate for  $\text{NH}_4^+$  turnover, and as the  $\text{NO}_3^-$  pool size divided by the gross nitrification rate for  $\text{NO}_3^-$  turnover.

## Results

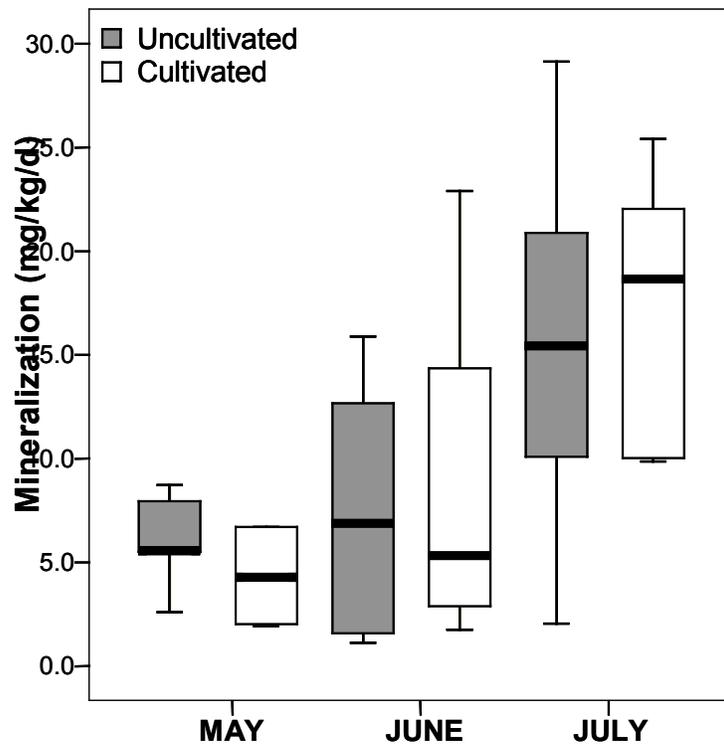
Soil  $\text{NH}_4^+$  levels in the uncultivated wetlands ( $\text{mg NH}_4^+\text{-N kg}^{-1}$  soil) were significantly higher than cultivated wetlands for all experimental dates (Fig. 1). Over the course of the growing season, there was a small increase in the amount of  $\text{NH}_4^+$  measured in the cultivated soils but no significant change in the uncultivated soils. This land use difference in soil  $\text{NH}_4^+$  levels was not reflected in gross mineralization levels. Gross mineralization levels increased similarly for both land uses from May to July (Fig. 2). The  $\text{NH}_4^+\text{-N}$  turnover time reflected the land use differences in soil  $\text{NH}_4^+$ , with average turnover times of 1-2 days for smaller  $\text{NH}_4^+$  pools in the cultivated soils and 2-5 days for the uncultivated soils. Turnover rates for both land uses tended to become more rapid through the season as both production and consumption rates increased (Table 1).

**Table 1.** Turnover rates for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools in cultivated vs. uncultivated wetlands.

		Days to turnover (S.D.)	
		$\text{NH}_4^+$	$\text{NO}_3^-$
<b>Uncultivated</b>	<b>May</b>	2.8 (1.0)	0.5 (0.4)
	<b>June</b>	4.9 (4.1)	1.5 (1.2)
	<b>July</b>	2.7 (2.9)	1.2 (0.6)
<b>Cultivated</b>	<b>May</b>	1.5 (1.1)	3.4 (1.0)
	<b>June</b>	1.6 (1.3)	1.8 (1.0)
	<b>July</b>	0.6 (0.3)	1.7 (1.0)

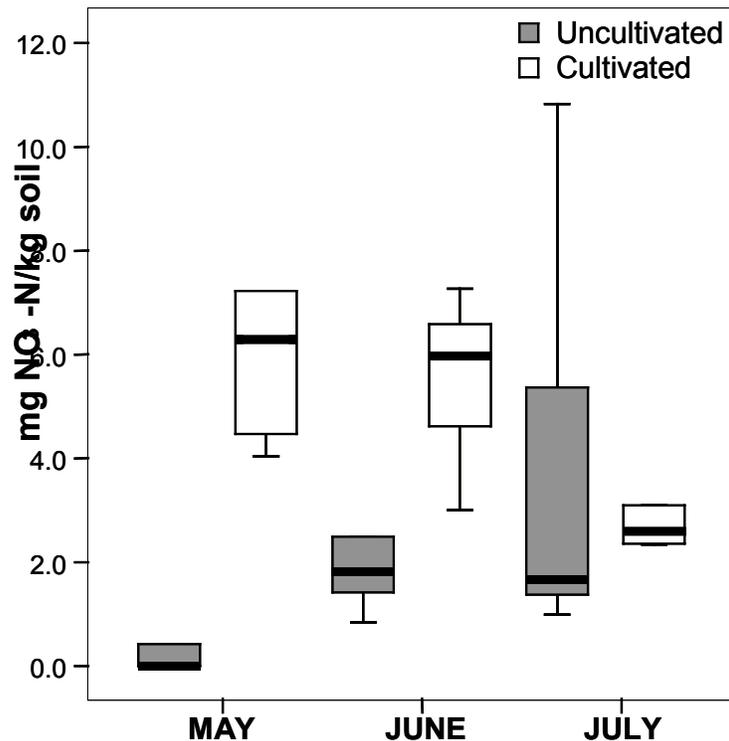


**Figure 1.** Soil  $\text{NH}_4^+$  levels in cultivated vs. uncultivated ephemeral wetlands.

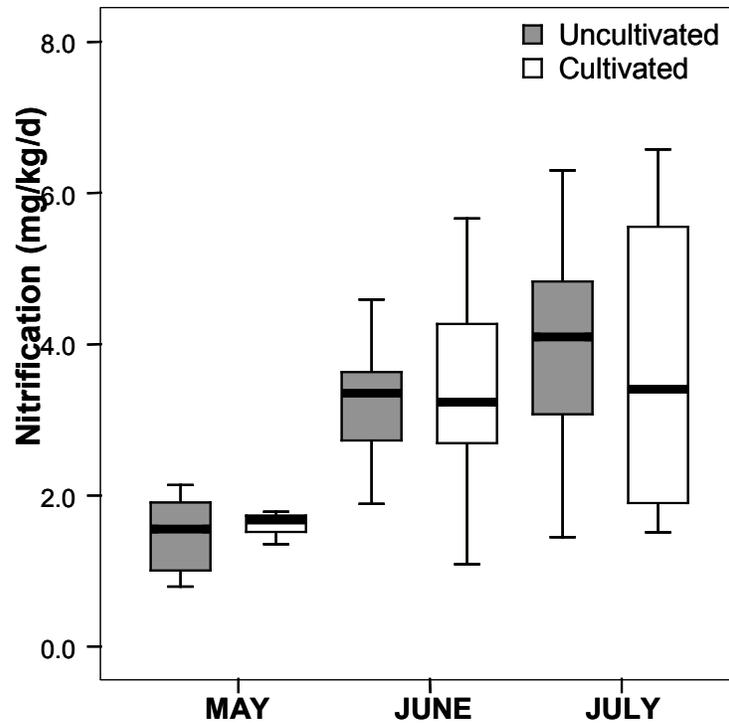


**Figure 2.** Gross  $\text{NH}_4^+$  mineralization rates in cultivated vs. uncultivated ephemeral wetlands.

The land use effect for soil  $\text{NO}_3^-$  ( $\text{mg NO}_3^- \text{-N kg}^{-1}$  soil) was inverse that observed for soil  $\text{NH}_4^+$ : cultivated soils had significantly greater soil  $\text{NO}_3^-$  than uncultivated soils (Fig. 3). Mean soil  $\text{NO}_3^-$  for cultivated soils was similar for May and June, but lower in July. Mean soil  $\text{NO}_3^-$  for uncultivated soils increased through the season, resulting in equal soil  $\text{NO}_3^-$  for both soils in July. There was no land use effect on gross nitrification levels and nitrification rates increased for both soils from May to July (Fig. 4). As was observed for  $\text{NH}_4^+$ , soil  $\text{NO}_3^-$  turnover reflected soil  $\text{NO}_3^-$  levels, with more rapid turnover ( $\leq 1$  d) occurring in the uncultivated soils with their lower soil  $\text{NO}_3^-$  levels (Table 1). However, unlike the seasonal decrease observed for the  $\text{NH}_4^+$  turnover,  $\text{NO}_3^-$  turnover became slower for the uncultivated soils (from 0.5 to 1.5 d) and more rapid for the cultivated soils (from 3.5 to 2 d) between the beginning and end of the growing season, reflecting soil  $\text{NO}_3^-$  levels over the same time period.



**Figure 3.** Soil  $\text{NO}_3^-$  levels in cultivated vs. uncultivated ephemeral wetlands.



**Figure 4.** Gross nitrification rates in cultivated vs. uncultivated ephemeral wetlands.

## Discussion

The N-production and -consumption rates reported for these ephemeral wetlands were within the range of previously-reported values for agricultural and grassland soils (Booth et al., 2005). However, there were significant differences in the size of the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools between cultivated and uncultivated soils despite similar gross mineralization and nitrification rates, suggesting mechanistic N-cycling differences under the two ecosystems. From the similar gross N-production rates, we can infer that neither mineralization nor nitrification is substrate-limited in either soil. From the different soil inorganic-N levels, however, we can infer that the fate or cycling mechanism for the produced N differs between soils.

For example, the higher levels of soil  $\text{NH}_4^+$  in uncultivated soils may indicate a greater occurrence of heterotrophic nitrification under the associated willow ring or riparian grassland vegetation. Uncultivated soils are more likely to have a significant fungal biomass and hence, a greater tendency towards heterotrophic nitrification whereby the biomass oxidizes both organic and inorganic substrate to produce  $\text{NO}_3^-$  (Paul and Clark, 1996). In contrast, the cultivated wetlands will be dominated by bacterial biomass and hence autotrophic nitrification will dominate. Therefore for both soils to produce the comparable amounts of  $\text{NO}_3^-$  measured in the gross nitrification experiment, nitrifiers in the cultivated soil would consume more  $\text{NH}_4^+$  whereas those in the uncultivated soil would consume both  $\text{NH}_4^+$  and organic N to meet their needs.

Similarly, differences in the size of the soil  $\text{NO}_3^-$  pool may be attributable to differences in  $\text{NO}_3^-$  consuming processes. There was significantly higher gross  $\text{NO}_3^-$  consumption in the uncultivated soils throughout the growing season and consumption rates increased at a rate

similar to gross nitrification rates, resulting in minimal increases in extractable soil  $\text{NO}_3^-$  through the season. Given the absence of plant uptake or leaching as a loss mechanism in the incubated cores,  $\text{NO}_3^-$  reduction is likely the primary pathway for uncultivated  $\text{NO}_3^-$  consumption. Based on  $^{15}\text{N}$  measurements of the  $\text{NH}_4^+$  extracted from  $^{15}\text{NO}_3^-$  labeled cores in the uncultivated soils, there was a small amount of dissimilatory nitrate reduction to ammonium (DNRA) taking place, but the dominant  $\text{NO}_3^-$  consumption mechanism was likely reduction by denitrification. Previous authors have also noted that  $\text{NO}_3^-$ -N does not tend to accumulate under native grass vegetation (Malo et al., 2005).

Dividing the N pool size by its corresponding gross N-production rate gives an estimation of how rapidly that pool turns over or replaces itself. For these soils, the smaller the pool, the more rapidly it turned over. The  $\text{NH}_4^+$  pool in the cultivated soils and the  $\text{NO}_3^-$  pool in the uncultivated soils both turned over in approximately 1 d, comparable to previously reported turnover rates in a range of soils (Booth et al., 2005). This rapid turnover further demonstrates the limited utility of a “snapshot” of extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  as indicators of either plant-available N for agricultural production or, in the case of  $\text{NO}_3^-$ , denitrification substrate for  $\text{N}_2\text{O}$  emissions.

There was a clear seasonal effect for gross N-producing and N-consuming processes. Booth et al. (2005) noted a strong positive relationship between mineralization,  $\text{NH}_4^+$  assimilation and nitrification rates across a range of ecosystems. A positive relationship was also noted in our soils when all data were considered. However, when examined on a date-by-date basis, the relationship did not persist. In these soils, the correlation was apparently more a function of a similar response of the N-producing processes to the increase in soil temperature through the growing season (Bengtson et al., 2005), with rates of both processes increasing from May to July. Booth et al. (2005) noted that the temperature-nitrification relationship depends in part on the availability of  $\text{NH}_4^+$ . Therefore, when gross mineralization increases with soil temperature, gross nitrification will also increase, as long as no other  $\text{NH}_4^+$ -assimilating or consuming process dominates. Schimel and Bennett (2004) suggest that N-producing, -consuming, and -emitting processes are most likely occurring in close proximity in soil microsites and are controlled in part by N availability. Nitrogen-producing and -consuming processes may be similarly activated by enzymes when the intracellular N content of associated microorganisms reaches critically low levels (Bengtson et al., 2005). Further investigation into the landform and land use impacts on these N-cycling feedback mechanisms is necessary.

## Conclusion

Land use has had a significant impact on the soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  levels in these ephemeral wetlands but no influence on gross mineralization and nitrification rates. Instead, the primary effect of land use on N-cycling processes appears to be in the mechanistic details, where each land use favors a different suite of processes carried out by a specific group of microorganisms. The assemblage of dominant microbial players present under a given land use appears to be more important than other environmental factors previously assumed to be decisive, including fertilization and WFPS. The complexity of the interrelationships among the N-producing, N-consuming, and N-emitting processes highlights the need for greater knowledge of the structure and lability of the soil organic N pool and of defining the microbial communities responsible for N cycling under different land use regimes.

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