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# Changes in Potential Nitrous Oxide Efflux during Grassland Restoration

Drew A. Scott,\* Steven T. Rosenzweig, Sara G. Baer, and John M. Blair

## Abstract

Nitrous oxide efflux from soil is an important ecological process in terms of global climate impacts, stratospheric chemistry, and soil fertility. The effects of grassland restoration on nitrous oxide ( $\text{N}_2\text{O}$ ) efflux in formerly cultivated agricultural soils are not well known. Restoration changes the storage and availability of soil C and N, with potential consequences for  $\text{N}_2\text{O}$  efflux. We examined changes in potential  $\text{N}_2\text{O}$  efflux across a 35-yr chronosequence of grassland restorations, using laboratory incubations at moisture levels that maximized  $\text{N}_2\text{O}$  emissions, to quantify the relationship between  $\text{N}_2\text{O}$  efflux and soil properties known to change predictably during grassland restoration. We found that restoring cultivated agricultural land to grassland rapidly decreased  $\text{N}_2\text{O}$  efflux from soils, although native prairie had a potential  $\text{N}_2\text{O}$  efflux higher than the agricultural land. The oldest restoration had  $\text{N}_2\text{O}$  efflux 50 times lower than native prairie. Changes in  $\text{N}_2\text{O}$  efflux were more strongly correlated with N mineralization than C mineralization, according to multiple regression analysis. These results suggest that grassland restoration could mitigate  $\text{N}_2\text{O}$  emissions for decades.

## Core Ideas

- Nitrous oxide efflux decreased rapidly during grassland restoration.
- Nitrous oxide was best predicted by N mineralization.
- The oldest restoration had  $\text{N}_2\text{O}$  efflux 50× lower than native prairie.

**N**ITROUS OXIDE ( $\text{N}_2\text{O}$ ) is a greenhouse gas with 298 times the warming potential of carbon dioxide and a precursor for catalytic ozone depletion in the stratosphere (Portmann et al., 2012; Myhre et al., 2013a,b). Emissions of  $\text{N}_2\text{O}$  also remove nitrogen (N) from the soil and reduce soil fertility (Tiedje, 1988; Ambus et al., 2001). Fertilized croplands are responsible for nearly 80% of anthropogenic  $\text{N}_2\text{O}$  emissions (Smith et al., 2007). As such, ceasing N fertilization should reduce  $\text{N}_2\text{O}$  emissions from soil as N becomes increasingly limited, but no studies have quantified potential  $\text{N}_2\text{O}$  flux in relation to changing pools of carbon (C) and N in soil during grassland restoration from agriculture.

Nitrous oxide is produced from microbial oxidation of ammonium and organic N (autotrophic and heterotrophic nitrification, respectively) and microbial reduction of nitrate to dinitrogen (denitrification; Firestone and Davidson, 1989). Nitrification, rather than denitrification, is a major source of  $\text{N}_2\text{O}$  in agricultural soils at 60% water-filled pore space (Pihlatie et al., 2004), but the primary source of  $\text{N}_2\text{O}$  emissions during grassland restoration from agriculture is unknown. Organic C serves as the electron source for denitrification, and nitrate serves as an alternate electron acceptor during anaerobic cellular respiration (Tiedje et al., 1984). Thus, the availability of organic C, labile organic N, and inorganic N can limit  $\text{N}_2\text{O}$  production from soil (van Groenigen et al., 2015). Because tillage depletes soil C pools,  $\text{N}_2\text{O}$  emissions often are limited by organic C availability in cultivated fields (Yeomans et al., 1992; Sotomayor and Rice, 1996). Never-cultivated prairies, however, have large stores of C but limited availability of inorganic N (Blair, 1997), and the latter is presumed to limit  $\text{N}_2\text{O}$  production from denitrification and nitrification (Sotomayor and Rice, 1996).

Grassland conversion to row-crop agriculture increases  $\text{N}_2\text{O}$  emissions from soils (Skiba et al., 1993; Ostrom et al., 2010), but the effect of restoring previously cultivated soils to grassland on  $\text{N}_2\text{O}$  efflux is less documented. Edaphic factors that influence  $\text{N}_2\text{O}$  emissions are known to change with grassland restoration from cultivated land. Soil aggregation increases, potential C mineralization and in situ  $\text{CO}_2$  efflux rates increase, microbial biomass C and N increase, and extractable inorganic N decreases during grassland restoration (Jastrow, 1996; Baer et al.,

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2002, 2003, 2010; Rosenzweig et al., 2016; Scott et al., 2017). The availability of inorganic N drives N<sub>2</sub>O efflux because conversion of ammonium and nitrate are primary sources of N for N<sub>2</sub>O emissions via nitrification and denitrification, respectively (Sutka et al., 2006). Aggregate structure plays a role in emissions because the formation of microaggregates within macroaggregates physically protects organic matter from mineralization (Six et al., 2000; Bimüller et al., 2016) that otherwise would provide a source of electrons for denitrification and creates microenvironments with distinct microbial communities (Kravchenko et al., 2014). However, aggregates also maintain anaerobic microsites that can allow denitrification to occur (Sexstone et al., 1985). Microbial immobilization of N as labile C pools increase from root inputs leads to progressive N limitation during grassland restoration on formerly cultivated soil (Baer et al., 2002, 2003; Baer and Blair, 2008; Rosenzweig et al., 2016). Microbial N demand increases as N is sequestered in long-lived soil organic matter, resulting in little N available to plants (Williams et al., 2001). However, it is unclear how shifting C and N availability during grassland restoration ultimately influences N<sub>2</sub>O efflux from soil.

We used a chronosequence of restored grasslands (i) to quantify potential N<sub>2</sub>O emission rates and (ii) to explore the relationship of N<sub>2</sub>O emission rates to soil variables known to influence nitrification and denitrification that change with restoration age (i.e., potential C mineralization, potential net N mineralization, microbial biomass C-to-N ratio, and percentage microaggregates-within-macroaggregates by mass [representing occluded organic matter]). We hypothesized that N<sub>2</sub>O efflux would decrease with restoration age as inorganic N availability decreases and physically protected soil C and N increase (Rosenzweig et al., 2016; Scott et al., 2017).

## Materials and Methods

### Study Site and Design

The restoration chronosequence consisted of prairies restored for 1, 3, 7, 15, and 35 y, an actively cultivated field, and a native (never-cultivated) prairie. This space-for-time substitution allowed us to evaluate effects of restoration age while minimizing temporal variability due to other factors (Kravchenko and Robertson, 2015). All fields were located within 2 km of each other on or adjacent to the Konza Prairie Biological Station and Long-Term Ecological Research site 9 km south of Manhattan, KS. Prior to restoration, all fields were managed with agronomic practices typical of the region. This would generally include crop rotations of *Triticum aestivum* L. (winter wheat), *Zea mays* L. (maize), *Sorghum bicolor* (L.) Moench ssp. *bicolor* (grain sorghum), or *Glycine max* (L.) Merr. (soybean) production. Inorganic N and P were added as recommended, depending on the specific crops planted. Mean annual precipitation is 835 mm and mean annual temperature is 12.7°C (15-yr average; Scott et al., 2017). All fields were on deep soil lowlands with negligible slope. *Andropogon gerardii* Vitman and *Sorghastrum nutans* (L.) Nash were the most frequently encountered species; species composition from August is provided in Scott et al. (2019). Fire history varied because sites were different ages and fire was not prescribed until following the third growing season, when fuel

loads were adequate. The timing of the most recent prescribed burn and soil properties are reported in Table 1.

The soil series were similar in the sampled A horizon. The 35-yr restoration (located 2 km from the Konza Prairie Biological Station) and native prairie were on a Clime silty clay loam (fine, mixed, active, mesic Udothentic Haplustolls)–Sogn silt loam complex (loamy, mixed, superactive, mesic Lithic Haplustolls) formed from shale and limestone residuum, respectively (Soil Survey Staff, 2014). Other sites were on Reading silt loam soil (fine silt, mixed, superactive, mesic Pachic Argiudoll) formed from alluvial silt deposits.

### Soil Sampling

We delineated four plots (4 by 4 m each) separated by a minimum 2-m buffer in an area with representative vegetation in each field. We took two 5.5-cm-diam. cores per plot to measure bulk density and aggregate structure and 10 composited 2-cm-diam. soil cores per plot to measure soil properties and processes (microbial biomass C and N, and potential C and N mineralization rates) to a depth of 0 to 10 cm in June 2013. Four intact cores for N<sub>2</sub>O efflux measurements were collected in 5-cm-diam. beveled polyvinyl chloride tubing to a depth of 10 cm in August 2013 from approximately the same locations as other soil cores (one core per plot). This sampling time was chosen because soil water content and net N mineralization are generally highest early in the growing season (Groffman et al., 1993) and up to 67% of N<sub>2</sub>O emissions occur in summer months (Bremner et al., 1980). Nitrous oxide cores remained in the polyvinyl chloride during laboratory incubations, to maintain an intact soil core.

### Laboratory Measurements

Intact soil cores used to measure N<sub>2</sub>O efflux were adjusted to 50% water-holding capacity, equivalent to approximately 60% water-filled pore space, to maximize N<sub>2</sub>O production. Field-collected soils adjusted to 50% water-holding capacity ranged from 50 to 78% water-filled pore space. Cores were placed into mason jars with a rubber septum for sampling headspace gas and incubated in the dark at 23°C. Any plants that emerged during incubation were removed with forceps. We measured N<sub>2</sub>O from headspace gas every 2 to 3 d for 16 d and calculated daily N<sub>2</sub>O efflux based on the difference at 3 and 6 d of incubation, while the N<sub>2</sub>O accumulation rate was linear. One milliliter of headspace gas was injected into a Shimadzu GC-2014 (Shimadzu Corp.) equipped with an electron capture detector.

**Table 1.** Soil properties in the top 10 cm (average of four plots) and time since most recent burn at each site.

Restoration age	pH	Total N	Total C	Time since most recent fire
yr		g m <sup>-2</sup>		
0	5.6	135.65	1603.82	N/A†
1	6.1	133.50	1537.07	N/A
3	6.2	133.62	1522.57	N/A
7	5.9	141.04	1471.15	4 yr ago
15	6.3	145.89	1635.05	Previous year
35	7.5	203.25	2603.02	Previous year
Native	6.5	279.88	3457.61	Previous year

† N/A, not applicable; fields not yet burned.

Microbial biomass C and N were determined by fumigation direct-extraction (Brookes et al., 1985) on composited soil samples adjusted to 50% water-holding capacity (as described in Rosenzweig et al., 2016). Potential net N mineralization rates were measured using short-term (28-d) aerobic laboratory incubation (Robertson et al., 1999; also described in Rosenzweig et al., 2016). To complement the laboratory-based potential C and N flux assays, we also measured 2 M KCl-extractable N in freshly-collected field soils and in situ CO<sub>2</sub> efflux; details of these procedures are described in Rosenzweig et al. (2016). Microaggregate-within-macroaggregate C and N were measured using a hierarchical wet sieving method (Six et al., 2000; full details are in Scott et al., 2017).

## Statistical Analyses

All statistical analyses were conducted with R (R Core Team, 2016). Multiple linear regression models to predict N<sub>2</sub>O efflux from intact cores were constructed using measures of C and N availability (microbial biomass C-to-N ratio, potential N mineralization, potential C mineralization, and percentage microaggregate-within-macroaggregate by mass). The chronosequence included one restoration field of each age; therefore, we used subplot data only in the exploratory multiple regression analysis (Jastrow, 1996). This was necessary for sufficient sample size to identify the best predictors of N<sub>2</sub>O and because our inference was aimed at explaining N<sub>2</sub>O efflux from the soil cores. All variables had a variance inflation factor <5, indicating no collinearity. Backward model selection was performed by dropping the worst-fitting variable, then selecting the model with the lowest Bayesian Information Criterion.

Linear and exponential decay and rise to maximum regression models (selected by Bayesian Information Criterion) for all variables were fit with time since restoration; cultivated and native prairie means are displayed with regression results for reference. A single N<sub>2</sub>O efflux value from the 1-yr restoration was a significant outlier according to a Grubbs' test, and that observation was omitted from our analyses. All regressions were performed with means of the dependent variable for each field. All analyses used  $\alpha = 0.05$ . For multiple comparisons between restoration ages, responses were considered significant if there were non-overlapping standard error bars.

## Results

Nitrous oxide efflux from soil decreased exponentially with increasing restoration age (Fig. 1A). Nitrous oxide efflux from the cultivated field and native prairie were similar, but restorations older than 15 yr had efflux values lower than native prairie (Fig. 1A). Potential N mineralization rate was the only significant predictor of N<sub>2</sub>O efflux rate ( $R^2 = 0.34$ ,  $P = 0.005$ ), which decreased rapidly with restoration age ( $t = 3.21$ ,  $P = 0.005$ ; Fig. 1B). Carbon mineralization increased rapidly with restoration age (Fig. 1B), whereas microbial biomass C-to-N ratio displayed no directional change (Fig. 1C).

## Discussion

In contrast to our prediction that N<sub>2</sub>O efflux would be limited primarily by C availability early in the restoration

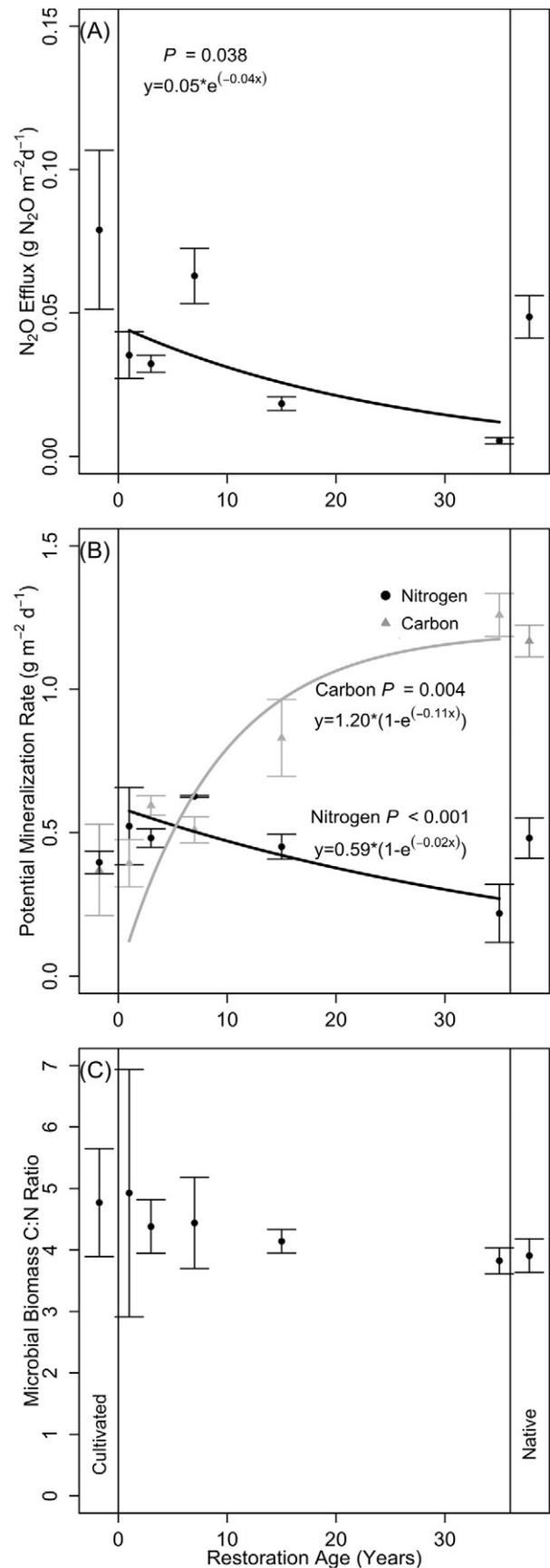


Fig. 1. Temporal patterns in (A) N<sub>2</sub>O efflux, (B) C and net N mineralization rates, and (C) microbial biomass C-to-N ratio along the restoration chronosequence. Mean values  $\pm$  SE for each field (representing a different restoration age) are plotted. Linear and exponential regressions were performed on means.

process, the exploratory regression model suggests that N availability, not C availability, corresponds with reduced N<sub>2</sub>O efflux during restoration. Surprisingly, the oldest restoration had N<sub>2</sub>O efflux values nearly 50 times less than native prairie. The high level of N<sub>2</sub>O efflux from native prairie corresponds with low available N but high net N mineralization rate, suggesting high N turnover. Additionally, a previous study found an order of magnitude higher nitrification (chlorate inhibition assay) and denitrification (short-term anaerobic assay) enzymes in native prairie compared with agricultural fields at this site (Groffman et al., 1993), which could explain the relatively high rates of N<sub>2</sub>O efflux in the native prairie soil compared with the restorations in our study. Declining N<sub>2</sub>O efflux rate across the restoration chronosequence may also be due to increasing organic matter (from increased plant inputs) coupled with increasing plant and microbial demand for N, two components of progressive N limitation (Baer and Blair, 2008). Progressive N limitation is expected to continue for several decades because total C in the oldest restoration remained ~1000 g m<sup>-2</sup> below the native prairie reference (Scott et al., 2017). Because N is sequestered in long-lived soil organic matter during grassland restoration, microbial demand for N is expected to increase, leaving progressively less N available to plants (Williams et al., 2001).

Our laboratory-based measures of potential N<sub>2</sub>O efflux were conducted at moisture levels optimal for both nitrification and denitrification. Although in situ fluxes cannot be estimated from potential fluxes, the latter may still be a good indicator of temporal changes during restoration. For example, the field-based extractable soil N and in situ C efflux patterns exhibited across the chronosequence (Rosenzweig et al., 2016; Fig 2) were similar to laboratory-based potential C and N mineralization rates. Assuming our potential N<sub>2</sub>O efflux rates reflect trends in in situ rates, this study suggests that restoring cropland to grassland could help mitigate greenhouse gas efflux from soil for at least a few decades until high N turnover, as seen in native prairie, develops.

## Conflict of Interest

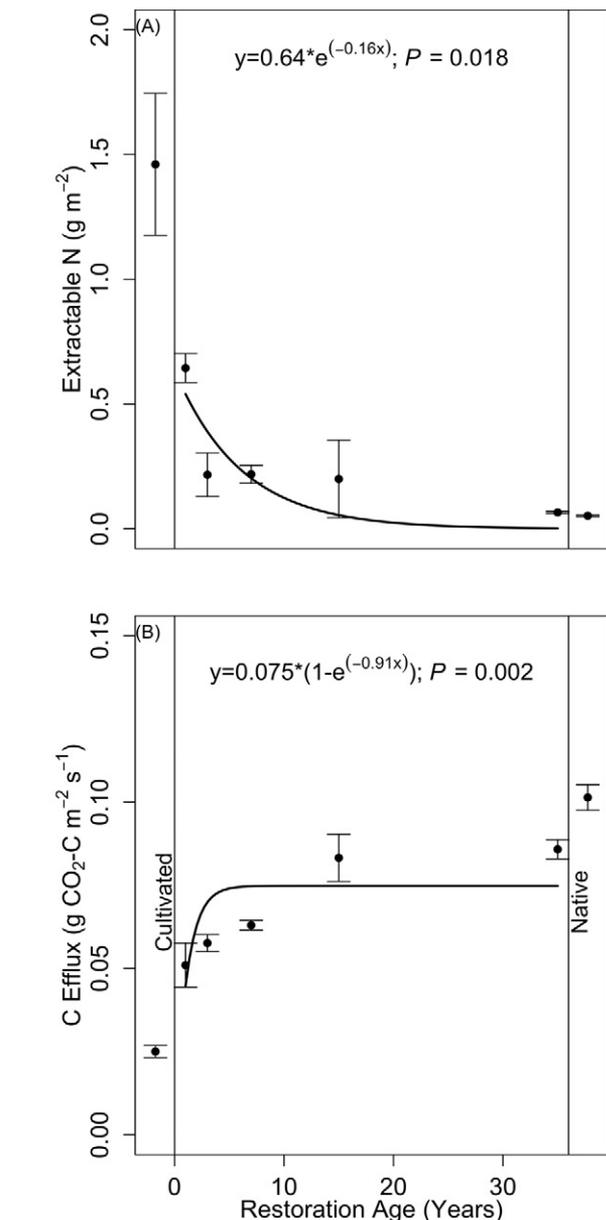
The authors declare no conflict of interest.

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**Fig. 2.** Temporal patterns in (A) extractable N and (B) in situ CO<sub>2</sub> efflux; modified from Rosenzweig et al. (2016) fit to exponential decay and exponential rise to maximum models, respectively.

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