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Nonlinear response of N_2O flux to incremental fertilizer addition in a continuous maize (*Zea mays* L.) cropping system

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Abstract

The relationship between nitrous oxide (N_2O) flux and N availability in agricultural ecosystems is usually assumed to be linear, with the same proportion of nitrogen lost as N_2O regardless of input level. We conducted a 3-year, high-resolution N fertilizer response study in southwest Michigan USA to test the hypothesis that N_2O fluxes increase mainly in response to N additions that exceed crop N needs. We added urea ammonium nitrate or granular urea at nine levels (0–292 $kg\,N\,ha^{-1}$) to four replicate plots of continuous maize. We measured N_2O fluxes and available soil N biweekly following fertilization and grain yields at the end of the growing season. From 2001 to 2003 N_2O fluxes were moderately low (ca. 20 $g\,N_2O\text{-}N\,ha^{-1}\,day^{-1}$) at levels of N addition to 101 $kg\,N\,ha^{-1}$, where grain yields were maximized, after which fluxes more than doubled (to $>50\,g\,N_2O\text{-}N\,ha^{-1}\,day^{-1}$). This threshold N_2O response to N fertilization suggests that agricultural N_2O fluxes could be reduced with no or little yield penalty by reducing N fertilizer inputs to levels that just satisfy crop needs.

Keywords: agriculture, ammonium, denitrification, maize, N availability, N fertilizer, nitrate, nitrification, nitrous oxide, soil nitrogen, threshold

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Introduction

Nitrous oxide (N_2O) is an important trace gas that contributes to the greenhouse effect and controls stratospheric ozone. At present, N_2O concentrations are increasing in the troposphere at a rate of 0.8 $ppb\,yr^{-1}$ (IPCC, 2001). Nitrification and denitrification are the principle natural sources of this gas to the atmosphere; during nitrification ammonium (NH_4^+) is converted to nitrate (NO_3^-) and during denitrification NO_3^- is reduced to N_2O and dinitrogen (N_2).

Soil N availability can have a major impact on N_2O fluxes across a range of ecosystem types. In forests, N_2O fluxes are usually higher in sites that have been fertilized by atmospheric deposition or experimental N addition (Hall & Matson, 1999; Erickson *et al*., 2001; Butterbach-Bahl *et al*., 2002), that have leguminous species (Erickson *et al*., 2001, 2002), or that have soils of high fertility (Matson & Vitousek, 1990). In agricultural systems, N fertilization increases N_2O fluxes relative to unfertilized controls (Saharawat & Keeney, 1986; Eichner, 1990; Davidson *et al*., 1996; Smith *et al*., 1998), and intensively cropped systems generally have higher fluxes than unmanaged systems on similar soils (Mosier *et al*., 1991; Robertson *et al*., 2000).

Agricultural soils contribute about 50% of the global anthropogenic N_2O flux (IPCC, 2001), which is equivalent to a global warming potential of 1.0 $Pg\,C\,yr^{-1}$ (Robertson, 2004). Current IPCC protocols calculate agriculture's contribution to atmospheric N_2O loading as a simple percentage of total N inputs: $1.25 \pm 1\%$ of added N is estimated to be lost as N_2O based on fluxes from fertilized vs. unfertilized field plots (Eichner, 1990; Mosier *et al*., 1998). Responses of N_2O fluxes to intermediate levels of fertilizer N are unknown.

Theory suggests that the relationship between N inputs and N_2O flux may be more complex, and in particular that N_2O flux may exhibit a threshold response to N inputs. Nitrogen often limits both plant growth and N_2O production in terrestrial ecosystems, so that where plants are competing with microbes for soil N, N_2O production will be suppressed until plant N demands have been fully satisfied. Moreover, if

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plants are better competitors for soil N than are N_2O-producing microbes, then N_2O fluxes will be relatively low until plant N demand diminishes, at which point N_2O production may jump as a result of greater N availability for microbes.

Hydrologic NO_3^- export from agricultural soils appears to exhibit threshold responses to N fertilizer additions. Bergstrom & Brink (1986) found a nonlinear response of NO_3^- leaching to increasing N additions (five levels of calcium nitrate) for an agricultural system that was dominated by cereal crops. Other studies have shown that the amount of NO_3^- leached from fertilized cropping systems is low until N fertilizer levels reach the point at which yields no longer increase, at which point NO_3^- leaching increases abruptly (Steinhilber & Meisinger, 1995; Andraski *et al*., 2000; Power *et al*., 2000).

If a nonlinear relationship between N_2O flux and soil N availability exists, a result would be the possibility of fertilizing at N rates that promote the dual goals of both high crop yield and low N_2O flux. Given the large contribution of farming systems to the global N_2O budget, defining N rates where yields are maximized and environmental harm minimized could benefit both food production and the environment.

From 2001 to 2003 we measured N_2O flux along a nine-point N fertilizer gradient in continuous maize to test the hypothesis that N_2O fluxes respond to fertilizer N inputs in a nonlinear fashion, and in particular that the fluxes are low until the point along the gradient where grain yields no longer increase.

Materials and methods

Site description

We conducted this study at the W. K. Kellogg Biological Station Long-Term Ecological Research (KBS LTER) site. KBS is located in southwest Michigan at the northern end of the US corn belt, 50 km east of Lake Michigan (42° 24′N, 85° 24′W, elevation 288 m) on soils developed from glacial outwash deposited 12 000 years ago. Soils are mainly of the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) series, which co-occur on our site (Collins & Crum, 1996). The area receives approximately 90 cm of precipitation annually, about half as snow, and the mean annual temperature is 9.7 °C. During the 3 years of this study, annual rainfall was 103.3 cm for 2001, 73.2 cm for 2002, and 88.5 for 2003. During years with normal precipitation, maize yields at KBS are typical of the North Central region as a whole. Further site and soils descriptions are available at http://lter.kbs.msu.edu.

From 2000 to 2003 we fertilized four replicates of continuous maize at nine levels of N (0, 34, 67, 101, 134, 168, 202, 246, and 291 kg N ha^{-1}) for a total of 36 plots. Plots were 5 by 30 m arranged in a randomized block design ($n = 4$ blocks). Fertilizer was applied in a split application; 34 kg N ha^{-1} as 28% urea ammonium nitrate (UAN) was injected 70 mm to the side of and 50 mm below the seed in all fertilized treatments at planting (9 May, 2001; 22 May, 2002; 30 May, 2003), and the remainder was injected as UAN between the rows in late June in 2001 and 2002 (28 June) or broadcast as granular urea and incorporated in mid-July in 2003 (14 July) when the maize was approximately 0.3 m high.

Other agronomic management followed best management practice (BMP) for area crops. Rows were planted at 0.76 m intervals at a density of 11 340 seeds ha^{-1}, resulting in six rows per plot. Chlorpyrifos was applied at 5.6 kg ha^{-1} to control European corn borer. S-metolachlor, mesotrione, and atrazine were applied to control weeds. In 2003, the plots were irrigated (ca. 5 cm) to make up the difference between 2003 rainfall and the 30-year seasonal mean.

N_2O analyses, N availability, and yield

From 2001 to 2003 we measured N_2O fluxes before each fertilization event, the day that fertilizer was applied (except in 2003), the day after fertilizer was applied, and then approximately biweekly until fluxes diminished (except 2001). N_2O measurements and analyses were conducted using a static-chamber method used for earlier studies at the site (e.g. Robertson *et al*., 2000). In 2001, one 2.8 L cylindrical chamber (15.5 cm diameter × 17 cm height) equipped with a septum was embedded 2 cm into the soil in each plot at the beginning of a flux determination and then removed after the measurements were completed. In 2002 and 2003, one chamber base made from PVC pipe was installed in each plot (25 cm diameter × 10 cm height) and remained in the field between agronomic operations, with lids attached only for measurement periods of up to 2 h. Four gas samples were taken through the rubber septum over the incubation period (<2 h) by transferring 5 mL headspace aliquots to 3 mL crimp-top vials that were then taken to the laboratory for analysis within 36 h. N_2O measurements were made in all four replicates of the nine different N levels for a total of 36 plots per sample date.

Gas samples (0.5 mL) were injected into a gas chromatograph (Hewlett Packard 5890 Series II, Rolling Meadows, IL, USA), where N_2O was separated on a Porapak QS column (1.8 m, 80/100 mesh, held at 80 °C) and quantified with a ^{63}Ni electron capture detector at 350 °C. Carrier gas was argon/methane (90/10).

Soil samples were collected for determination of available N and soil moisture content after each gas sampling from all treatments in two replicates in 2002, from four replicates after harvest in 2001 and 2002, and from four replicates prior to planting in 2002. Soils were sampled to 25 cm within and between plant rows (eight subsamples) and composited. Before analysis, samples were sieved to 4 mm, subsampled for moisture determination, and 10 g were extracted in 100 mL of 1 N KCl for inorganic N analysis. Extracts were shaken and allowed to equilibrate for 24 h, when they were shaken again, allowed to settle for 1 h, and then filtered (Pall Life Sciences, Type A/E Glass Fiber Filter, Ann Arbor, MI, USA). Filtered extracts were frozen until analyzed for NH_4^+ and NO_3^- using an Alpkem 3550 Flow analyzer (OI Analytical, College Station, TX, USA). Soils for gravimetric moisture determination were dried for 48 h at 60 °C.

Maize yields were determined using plot-size combines. On 9 November, 2001; 17 October, 2002; and 10 November, 2003 two center rows were harvested in each plot. Reported yields are adjusted to standard grain moisture content of 15%.

Data analysis

N_2O flux and total soil inorganic N data were log transformed and then analyzed using one-way ANOVA in SYSTAT 7.0 after testing for block effects. Chamber means were used in the analysis to avoid issues with repeated measures of the same experimental unit. Grain yields across the N addition gradient were analyzed using PROC NLIN in SAS 8.0. Piecewise regression (Makowski *et al.*, 2001) was used to generate a model that accurately described the yield response to increasing N additions (a linear increase in the 0–101 kg N ha^{-1} region, an asymptote at $\sim$ 101 kg N ha^{-1}, and a plateau to describe the 101–291 kg N ha^{-1} region). From this regression analysis we defined the point along the N addition gradient where yields stopped increasing and determined the greatest average yield. We interpolated linearly between the sampling dates to calculate flux rates for each day and then totaled the daily rates for the entire sampling period to estimate the total N exported as N_2O.

Results

From 2001 to 2003, fluxes differed significantly across the N gradient ($P<0.0003$ for all three years). N_2O fluxes were low ($\sim$ 20 g N ha^{-1} day^{-1}) over the 0–101 kgN ha^{-1} fertilizer additions. For all years, at a fertilizer level of 134 kg N ha^{-1} N_2O fluxes increased sharply to $>$50 g N ha^{-1} day^{-1}, and then declined somewhat in the 168 and 202 kg N ha^{-1} treatments (Fig. 1). Grain yields in all three years increased in response to N additions from 0 to 101 kg N ha^{-1} and then leveled off at larger additions of N (Fig. 2).

The relationship between N_2O flux and yield shows lower fluxes associated with lower yields and higher

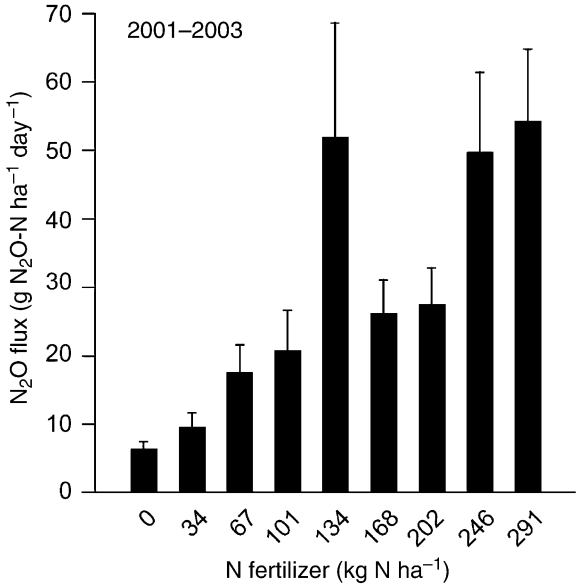

Fig. 1 Nitrous oxide fluxes across an N addition gradient in continuous maize in 2001, 2002, and 2003. Error bars represent standard error.

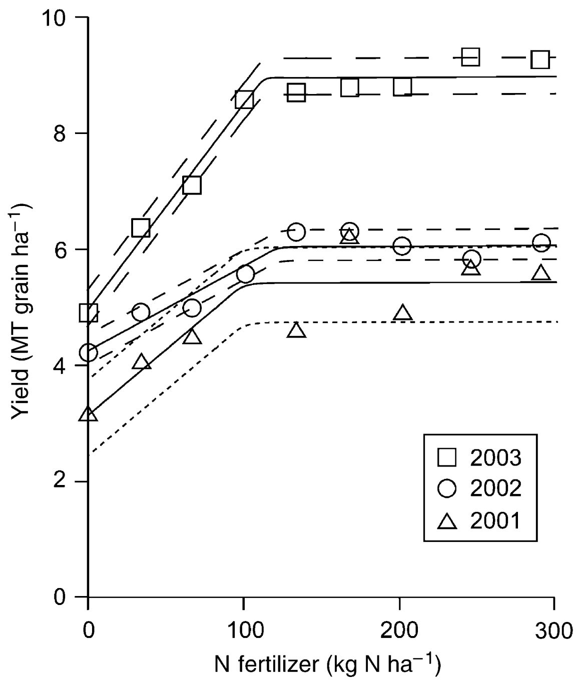

Fig. 2 Maize grain yield across the N addition gradient for 2001, 2002, and 2003. Dashed lines represent standard error.

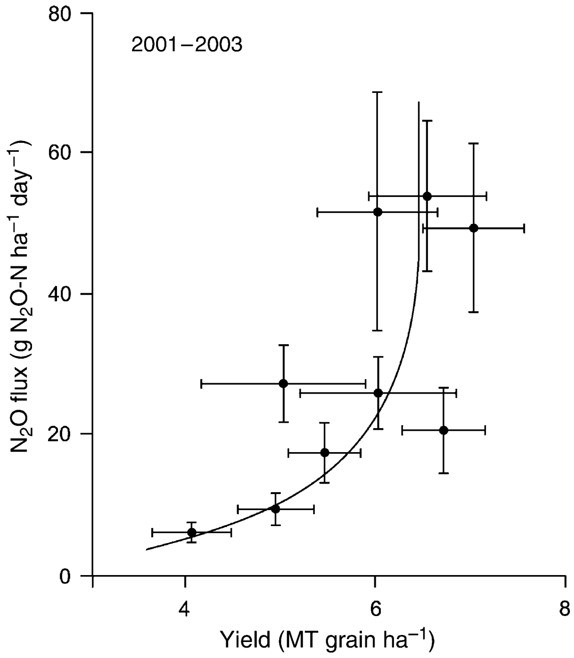

Fig. 3 Relationship between maize grain yield and nitrous oxide flux for the nine levels of N addition for 2001 ($r^2 = 0.739$), 2002 ($r^2 = 0.918$), and 2003 ($r^2 = 0.977$). Error bars represent standard error.

fluxes once yields were maximized (Fig. 3). For the 2002 data, we integrated between flux measurements to determine the total amount of N_2O-N that was exported from each of the treatments and calculated the proportion of fertilizer N applied that was lost. We found that a relatively constant percentage of the added fertilizer N was lost as N_2O in our first three N additions (34–101 kg N ha^{-1}). The greatest percentage of fertilizer N lost as N_2O (7%) occurred at 134 kg N ha^{-1}, and for N additions greater than 134 kg N ha^{-1} the proportion of N leaving as N_2O dropped to 2–4%.

In two years of this study yields were slightly lower than normal for Kalamazoo County (5.9 MT ha^{-1} for 1970–2000; USDA, 2000) and in one year it was higher. In 2001, 2002, and 2003 the average maximum yields were 5.4, 6.0, and 9.0 MT grain ha^{-1}, respectively. Distribution of rainfall through the growing season varied across the three years that we studied N_2O fluxes (Fig. 4). The corn was visibly water stressed for much of July in both 2001 and 2002 and we irrigated in 2003 to ensure that the crop was not water stressed.

Over the course of the 2002 growing season, N_2O fluxes varied by an order of magnitude. Immediately following fertilization, when N availability was highest, fluxes remained low (Fig. 5 top). Soil conditions at the time were dry because the last rain event (> 10 mm) had occurred 25 days previously (Fig. 5 middle). Our largest N_2O fluxes were associated with two rainfall events that

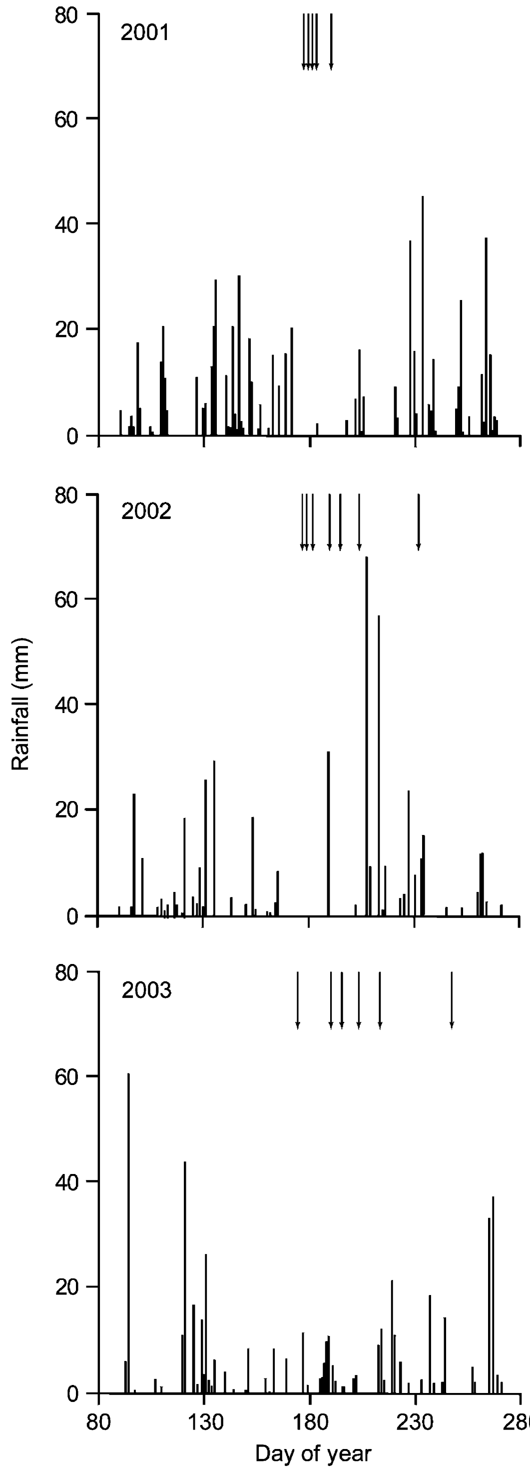

Fig. 4 Rainfall from 1 April to 30 September for the years 2001, 2002, and 2003. Arrows represent sampling dates (for 2003 one date in early October was excluded). For 2002, day of year 182, 204, and 232 were the sampling dates with gravimetric soil moisture > 15 g H_2O 100^{-1} g soil. In 2003, irrigation occurred on day of year 198 (12.5 mm), 205 (25.4 mm), 230 (17.5 mm), 237 (27.9 mm), and 254 (19 mm).

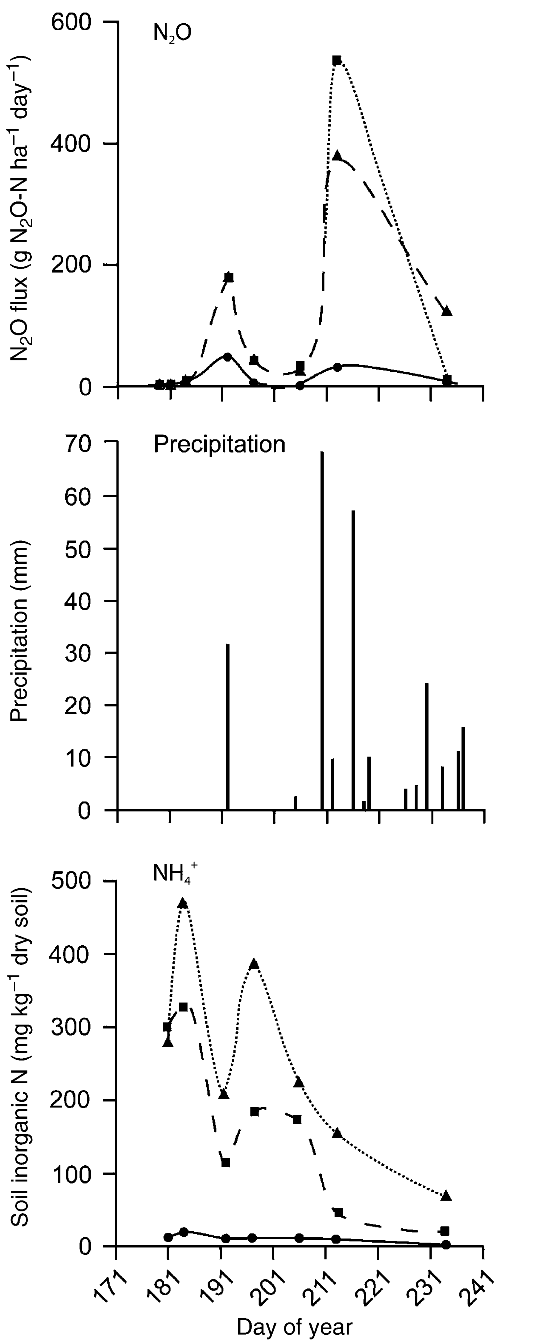

Fig. 5 Temporal sequence of nitrous oxide (N_2O) flux (top panel), precipitation (middle panel), and soil NO_3^- and NH_4^+ (bottom panel) for the sampling period for 0 (solid line), 134 (long dashed line), and 292 (dotted line) $kg\,N\,ha^{-1}$ rates of N addition.

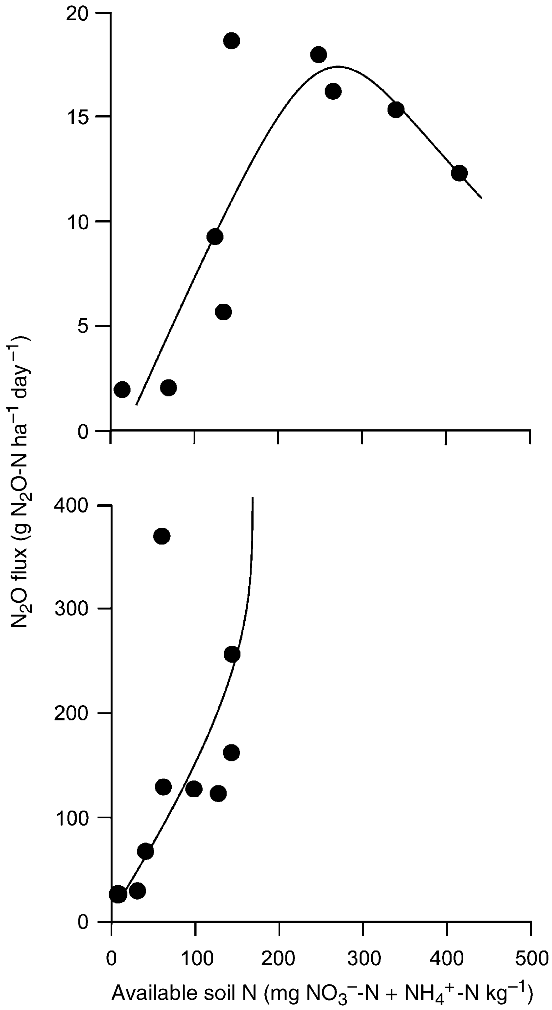

Fig. 6 Relationship between nitrous oxide flux and soil N availability on dry days (soil moisture < 15%; upper panel) and wet days (soil moisture 15%; lower panel).

occurred on 9 July (31 mm) and 27 July (68 mm), when N availability was still elevated (Fig. 5 bottom).

On dry sampling days (soil moisture <15%), N_2O fluxes exhibited a curvilinear response to increasing N availability to a point after which N_2O flux decreased somewhat (Fig. 6 top), and the magnitude of the response was much smaller than for wet days (Fig. 6). Under more mesic conditions (soil moisture >15%), N_2O fluxes exhibited a similar response to increasing N at lower N availability, but increased to a higher maximum rate (Fig. 6 bottom).

Discussion

N_2O fluxes were strongly affected by the amount of N fertilizer added and appeared to exhibit a nonlinear response in all three years of our study. For fertilizer additions up to $101\,kg\,N\,ha^{-1}$, fluxes remained at or below $20\,g\,N\,ha^{-1}\,day^{-1}$, with higher fluxes at greater additions of N. Yields behaved similarly, with production maximized at $101\,kg\,N\,ha^{-1}$, despite differences among years in the amount and distribution of rainfall or irrigation. Taken together, these data suggest the possibility for managing for high crop yield without generating larger N_2O fluxes (Fig. 3).

In order for the relationship between N_2O fluxes and grain yield to be useful for management, these patterns need to be consistent from year-to-year and representative of the local area. The distribution of N_2O fluxes across our N addition gradient was similar in 2001, 2002, and 2003 despite substantial differences in rainfall and the use of irrigation in 2003. Additionally, grain yield reached a maximum value at the same rate of N addition in all years of this study. Nitrogen rate studies on local farms, which considered somewhat different N levels (42, 87, 132, 177, 222, and 266 kg N ha^{-1}), show that greatest regional yields occur at 132 kg N ha^{-1} (MSUE, 2003), a point just below our threshold for N export as N_2O (Fig. 3). Grain yields in our study were just below average yields for Kalamazoo County in 2001 and 2002 and above average yields in 2003, which is not unexpected given that we irrigated in 2003 (USDA, 2001–2003). Consistency in key points along the N addition gradient, specifically where yields are maximized (101 kg N ha^{-1}) and N_2O export is high (134 kg N ha^{-1}), increase our confidence that a slight decrease in fertilization rate (in our case to 101 kg N ha^{-1}) can maintain yields while decreasing N_2O flux.

The distribution of N_2O fluxes across our N addition gradient appears to be driven by the patterns of soil N availability, while the *magnitude* of the fluxes appears to be controlled by the interaction between soil water content and N availability (Fig. 5). The requirement for the co-occurrence of increased N availability and soil water content for large N_2O fluxes is consistent with past studies in fertilized tropical agriculture (Davidson *et al*., 1996; Weier, 1999; Weitz *et al*., 2001), in tropical agroforestry with leguminous trees (Dick *et al*., 2001), in temperate agriculture during cover crop decomposition (Shelton *et al*., 2000), in farm fields in Great Britain (Ball *et al*., 2002; Dobbie & Smith, 2003) and Japan (Kusa *et al*., 2002; Hou & Tsuruta, 2003), and in cultivated boreal soils (Maljanen *et al*., 2002). In fact, the corequirement for increased soil water content and soil N availability for substantial N_2O fluxes may be more general, as it has been demonstrated in forested and grassland systems as well (e.g. Abbasi & Adams, 2000; Maddock *et al*., 2001; Ball *et al*., 2002; Maljanen *et al*., 2002). Our work demonstrates more precisely the point along the N availability gradient at which N_2O is exported when sufficient water is available, information that will be useful for ecosystems that are fertilized with N.

Emission factors

The proportion of N added that was lost as N_2O from our continuous maize ecosystem was 2–7% of N fertilizer, which falls within the 0.0–7.8% range found in other studies (as reported by Bouwman, 1996). For N additions up to 134 kg N ha^{-1} the pattern is consistent with the notion that at lower soil N availability crop uptake is a stronger sink for available N than are soil microbial processes. The fact that there is a small but linear increase in N_2O fluxes for N additions from 0 to 101 kg N ha^{-1} implies that the microbes have access to N somewhere in the soil system. Because yields stop increasing at N additions greater than 101 kg N ha^{-1}, we might expect that a greater percentage of the N applied will be exported as N_2O because crop demands have been met, leaving more N for nitrifiers and denitrifiers. Moreover, root exudates might further stimulate soil heterotroph activity at higher rates of crop production, which could stimulate denitrifiers (Robertson, 2000).

At N additions greater than 134 kg N ha^{-1} the proportion of N leaving as N_2O consistently dropped to its lowest level across the entire N gradient, perhaps indicating a change in the processes controlling N availability or N_2O production. At 134 kg N ha^{-1} there may have been a change in the microbial process that produced N_2O. If denitrification was the dominant source of N_2O, then the end product may have changed to NO or N_2, whereas if nitrification was the dominant source, then NO may have become a more important end product. A final possibility is that another N sink, such as microbial immobilization or luxury consumption by the crop, may be competing for N at these concentrations.

Our results suggest that a set emission factor is appropriate only when crops are fertilized at rates less than or equal to those required for maximum yields, as the percentage of fertilizer N that is leaving as N_2O becomes more variable at higher rates, even on the same soil and in the same crop (Fig. 1). Characterizing N_2O response thresholds for other crops, management practices, and rainfall regimes as well as understanding the underlying mechanisms for the patterns that we have described will be critical for mitigation of N_2O fluxes from agricultural fields through better management.

Conclusions

In our continuous maize ecosystem N_2O fluxes exhibited a nonlinear response to incremental additions of N fertilizer. N_2O fluxes were low until N additions reached 134 kg N ha^{-1}, at which point fluxes increased sharply. Increases in maize yields leveled off at 101 kg N ha^{-1}. It follows that we should be able to decrease N_2O fluxes in conventional agriculture by using less N fertilizer without imposing a large yield penalty.

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