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# **RESEARCH ARTICLE**

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#### **Key Points:**

- Topographic depressions did not consistently act as nitrous oxide hot spots, with controls on fluxes varying over the growing season
- Cold spring temperatures limit soil respiration that depletes dissolved oxygen to induce nitrous oxide production by denitrification
- Large summer rainfall events may not stimulate soil nitrous oxide emissions, even from topographic depressions, when soil moisture is low

#### Correspondence to:

A. H. Krichels, krichel2@illinois.edu

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# Dynamic Controls on Field-Scale Soil Nitrous Oxide Hot Spots and Hot Moments Across a Microtopographic Gradient

Alexander H. Krichels<sup>1</sup> 🕩 and Wendy H. Yang<sup>1,2,3</sup> 🕩

<sup>1</sup>Program in Ecology, Evolution, and Conservation Biology, University of Illinois, Urbana, IL, USA, <sup>2</sup>Department of Plant Biology, University of Illinois, Urbana, IL, USA, <sup>3</sup>Department of Geology, University of Illinois, Urbana, IL, USA

**Abstract** Soil nitrous oxide  $(N_2O)$  emissions are highly variable in space and time, making it difficult to estimate ecosystem level fluxes of this potent greenhouse gas. While topographic depressions are often evoked as permanent N<sub>2</sub>O hot spots and rain events are well-known triggers of N<sub>2</sub>O hot moments, soil N<sub>2</sub>O emissions are still poorly predicted. Thus, the objective of this study was to determine how to best use topography and rain events as variables to predict soil N2O emissions at the field scale. We measured soil N<sub>2</sub>O emissions 11 times over the course of one growing season from 65 locations within an agricultural field exhibiting microtopography. We found that the topographic indices best predicting soil N<sub>2</sub>O emissions varied by date, with soil properties as consistently poor predictors. Large rain events (>30 mm) led to an N<sub>2</sub>O hot moment only in the early summer and not in the cool spring or later in the summer when crops were at peak growth and likely had high evapotranspiration rates. In a laboratory experiment, we demonstrated that low heterotrophic respiration rates at cold temperatures slowly depleted soil dissolved O<sub>2</sub>, thus suppressing denitrification over the 2-3 day timescale typical of field ponding. Our findings show that topographic depressions do not consistently act as N<sub>2</sub>O hot spots and that rainfall does not consistently trigger N<sub>2</sub>O hot moments. We assert that the spatiotemporal variation in soil N<sub>2</sub>O emissions is not always characterized by predictable hot spots or hot moments and that controls on this variation change depending on environmental conditions.

**Plain Language Summary** Soils are the primary source of nitrous oxide ( $N_2O$ ), a greenhouse gas that contributes to global warming. Nitrous oxide is produced under oxygen-depleted conditions that can occur when soils are saturated with water. Large rain events are therefore thought to lead to short periods of high soil  $N_2O$  emissions. Landscape topography can shape spatial patterns in soil moisture and other soil properties that may regulate spatial variation in soil  $N_2O$  emissions. The goals of this study were to determine which topographic factors best predict spatial variation in soil  $N_2O$  emissions and under what conditions, and to determine when large rain events may not lead to bursts of soil  $N_2O$  emissions. We found that throughout the growing season, all topographic indices were poor predictors of soil  $N_2O$  emissions, but large rain events did not trigger similar pulses in the cool spring months or later in the summer when the crops were at peak growth with high soil water demand. This has important implications for predicting how soil  $N_2O$  emissions will respond to expected future changes in temperature and rainfall, feeding back on climate change.

# 1. Introduction

Soil nitrous oxide ( $N_2O$ ) emissions are highly variable in space and time, making it difficult to estimate ecosystem level fluxes of this potent greenhouse gas. High rates of  $N_2O$  emissions occur from discrete areas in space (hot spots) and short periods of time (hot moments) that disproportionately contribute to cumulative landscape scale  $N_2O$  emissions (Bernhardt et al., 2017; Groffman et al., 2009; McClain et al., 2003). The majority of  $N_2O$  emitted from soils is derived from denitrification (Ciais et al., 2013), an anaerobic process in which nitrate ( $NO_3^-$ ) is sequentially reduced to  $N_2O$  and then to inert nitrogen gas ( $N_2$ ; Knowles, 1982). Under increasingly anaerobic conditions coupled to high C availability, the proportion  $N_2$  to  $N_2O$ produced from denitrification increases, potentially limiting net  $N_2O$  emissions to the atmosphere (Chapuis-Lardy et al., 2007; Knowles, 1982). Rain events can saturate soils with water, facilitating a

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Topographic depressions can be characterized using a variety of approaches that relate differently to how N<sub>2</sub>O hot spots are formed. Light detection and ranging (LIDAR) data can be used to create fine-scale digital terrain models (DTMs) that predict patterns in soil moisture as a result of gravity-driven lateral transport of water (Fink & Drohan, 2016; Li et al., 2018; Turner et al., 2016). However, absolute elevation may not predict N<sub>2</sub>O hot spots in local depressions, which might accumulate water after relatively small rain events. Topographic fill models derived from DTMs can identify these discrete, localized areas lower in elevation relative to the surrounding terrain. Alternatively, soil properties can record a history of hydrological flow paths driven by topography (Burke et al., 1989; Schimel et al., 1985; Suriyavirun et al., 2019) and also incorporate factors beyond the role of hydrological flow in forming N<sub>2</sub>O hot spots. For example, clay transport downslope into depositional areas can lead to the development of clay-rich soils with high water retention conducive to sustaining hypoxic denitrification hot spots (Fissore et al., 2017). Soil magnetic susceptibility, which results from magnetic mineral dissolution in response to prolonged exposure to anaerobic conditions (Grimley et al., 2004), could also act as a direct record of historical soil redox regimes that could shape microbial communities with different functional potentials (DeAngelis et al., 2010; Pett-Ridge et al., 2006; Suriyavirun et al., 2019). While there are many approaches to predicting the locations of N<sub>2</sub>O hot spots, past studies have focused on using indices derived from DTMs, which have yielded only modest predictive power (Li et al., 2012; Turner et al., 2016).

Topography-mediated soil drainage can also indirectly alter soil N<sub>2</sub>O emissions by changing soil properties that directly control denitrification dynamics. Carbon (C) can accumulate in depressional areas through erosion (Nitzsche et al., 2017) or as a result of slowed decomposition under hypoxic conditions (Linn & Doran, 1984). Carbon compounds are oxidized during the heterotrophic process of denitrification, so higher C concentrations may lead to enhanced denitrification rates in depressional areas. Indeed, soil C concentrations are often correlated to  $N_2O$  emissions at the field scale (Li et al., 2012; Milne et al., 2011; Velthof et al., 1996; Yanai et al., 2003). Repeated saturation of soils can also lead to increases in pH (De-Campos et al., 2009), creating a variation in pH across topographic gradients (Suriyavirun et al. 2019). Soil pH also exerts a strong effect on denitrification dynamics, with higher N<sub>2</sub>O emissions observed in slightly acidic soils (Knowles, 1982; Milne et al., 2011; Russenes et al., 2016; Yanai et al., 2003). However, the effects of pH on N<sub>2</sub>O dynamics are complicated as elevated pH leads to increased denitrification rates and also increases the proportion of N<sub>2</sub>O that is reduced to inert N<sub>2</sub> (Knowles, 1982). While other factors can lead to spatial variation in soil organic C and pH, topography may be the most important factor driving this variability in homogenously managed agricultural fields. Uneven distribution of soil C and pH across topographic gradients may accentuate hot spots of denitrification following rain events and could drive spatial patterns in N<sub>2</sub>O emissions in-between rain events when topography does not lead to patterns in soil moisture.

Although topographic depressions are often characterized as  $N_2O$  hot spots (Ambus & Christensen, 1994; Saha et al., 2017; Turner et al., 2016; Yanai et al., 2003), they may not always act as hot spots in response to large precipitation events. In some cases, depressional areas may only act as hot spots in between rather than after rain events, with upslope areas acting as postrain hot spots (Krichels et al., 2019). This has been observed throughout the growing season under a range of soil inorganic N concentrations (Krichels et al., 2019). Rather than depleted soil  $O_2$  and increased soil C availability driving hot spots of denitrification in topographic depressions (Li et al., 2012; Milne et al., 2011; Turner et al., 2016; Velthof et al., 1996; Yanai et al., 2003), differences in soil biotic and abiotic properties may cause depressional and upslope areas to have distinct controls over denitrification rates (Krichels et al., 2019; Suriyavirun et al., 2019). For example, microbial community composition can differ between soils that experience different fluctuations in soil  $O_2$  concentrations (DeAngelis et al., 2010). Given that the composition of denitrifying



microbial communities can affect denitrification rates (Graham et al., 2014, 2016; Philippot & Hallin, 2005), differing denitrifier communities in depressional versus upslope areas may have distinct responses to precipitation events (Suriyavirun et al. 2019; Krichels et al. 2019). Furthermore, upslope soils can have a greater proportion of macroaggregates compared with depressional soils (De-Campos et al., 2009; Horn & Smucker, 2005; Six et al., 2004), potentially connecting aerobic and anaerobic soil microsites following large rain events (Algayer et al., 2014; Askaer et al., 2010; Sey et al., 2008) and allowing aerobic nitrification to continuously supply  $NO_3^-$  to fuel nearby denitrifiers (Krichels et al., 2019; Palta et al., 2016) via coupled nitrification-denitrification (Baldwin and Mitchell, 2000; Kool et al., 2010). Together, these factors can act as soil drainage legacy effects that cause the location of N<sub>2</sub>O hot spots within a field to be dynamic in response to recent precipitation.

Rain events are often thought of as triggers for hot moments of N2O emissions to occur across landscapes and in depressional areas (Dobbie & Smith, 2003; Molodovskaya et al., 2012; Sexstone et al., 1985), especially in agricultural fields where inorganic N concentrations remain high throughout the growing season (Sebilo et al., 2013). However, seasonal changes in temperature and crop growth could constrain this response. For example, depressional areas may not act as hot spots of denitrification in the cold springtime because cold conditions can prevent the depletion of  $O_2$  dissolved in soil pore water. In order to create hypoxic conditions that stimulate denitrification, microbial respiration must consume O<sub>2</sub> dissolved in soil pore water (Estop-Aragonés et al., 2013; Takai & Kamura, 1966). While large rain events can flush O<sub>2</sub> out of soil pores in temperate upland soils (Jarecke et al., 2016), particularly in depressional areas (Krichels et al. 2019), dissolved O<sub>2</sub> may persist in inundated soils under cold conditions in the spring when soils are still cold because rates of microbial respiration are highly sensitive to soil temperature (Davidson et al., 2012). In general, microbial processes are also likely to be inhibited under cold temperatures, including denitrification (Goodroad & Keeney, 1984; Holtan-Hartwig et al., 2002; Maag & Vinther, 1996), which may further limit N<sub>2</sub>O production in response to spring precipitation. Furthermore, microbial community composition can change seasonally in response to changes in temperature (Andrews et al., 2000; Bradford et al., 2008; Zogg et al., 1997) and crop growth (Buyer et al., 2002; Mbuthia et al., 2015), and denitrifying microorganisms may be more active at different points of the growing season. As such, the activation of hot spots of  $N_2O$ emissions may depend on when in the growing season large rain events occur.

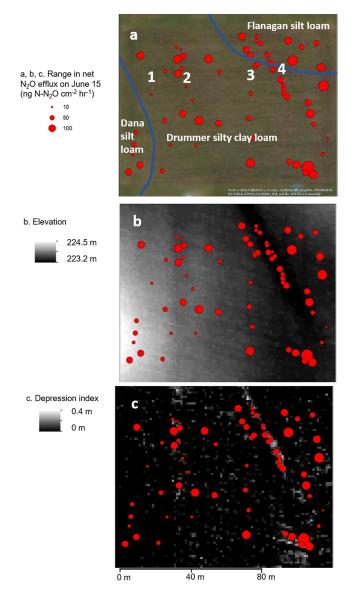
Although topographic depressions and rain events are generally considered major drivers of N<sub>2</sub>O hot spots and hot moments, it is still challenging to predict much of the spatiotemporal variation in net soil-atmosphere N<sub>2</sub>O emissions (Groffman et al., 2009). To better predict N<sub>2</sub>O hot spots and hot moments, we suggest adopting a dynamic view of where hot spots and when hot moments of soil N<sub>2</sub>O emissions occur as opposed to a static view of depressions such as hot spots and rain events as triggers for hot moments. To move toward this goal, we tested the following hypotheses: (1) the topographic index that best predicts spatial variation in  $N_2O$  emissions depends on recent rainfall amount, and (2) large rain events do not activate hot moments of N2O fluxes under relatively cold temperatures that slow heterotrophic consumption of oxygen in soil pore water, even when soil inorganic N concentrations are not limiting. To test the first hypothesis, we measured N<sub>2</sub>O emissions from 65 locations throughout an agricultural field before and after rain events over the course of a growing season, with a total of 11 sampling dates. To test the second hypothesis, we measured N<sub>2</sub>O emissions, heterotrophic respiration rates, and soil O<sub>2</sub> concentrations in inundated soil cores under warm and cold conditions. The temperature treatments were also assessed in factorial combination with a nitrate treatment (ambient versus amended) to account for the role of nitrate limitation of denitrification in driving the observed soil N<sub>2</sub>O emissions. This experiment was performed on intact soil cores collected from upslope and depressional areas of the field to assess if these areas of the field have different controls over soil N<sub>2</sub>O emissions (i.e., to account for historical drainage legacy effects). Furthermore, the experiment was conducted both early and late in the growing season to account for effects of seasonal changes in microbial community composition.

#### 2. Methods

# 2.1. Study Site

The study site was located at the University of Illinois at Urbana Champaign (UIUC) Energy Farm in Urbana, Illinois ( $40^{\circ}03'58.9''$ N,  $88^{\circ}11'28.2''$ W). Specifically, the study took place in a  $120 \times 100$  m field





**Figure 1.** Soil series classifications (a), elevation (b), and localized depressions (c) of the  $100 \times 20$  m study field in the UIUC Energy Farm. Scaled red dots represent magnitude of N<sub>2</sub>O emissions from each of the 65 chambers on 15 June 2017, the day after a 32 mm rain event. The numbers indicate the locations where soil O<sub>2</sub> concentrations were measured within the field and correspond to the data in Figure 3.

that is managed under annual rotation between corn (*Zea mays*) and soybeans (*Glycine max*). The site is fertilized with 203 kg·N·ha<sup>-1</sup> urea in the spring when *Z. mays* is planted, and it is not fertilized when *G. max* is planted. The field was planted with *G. max* during the 2017 growing season when this study took place. We chose to conduct this study in a field planted with *G. max* as *G. max* associates with nitrogen (N) fixing bacteria that can maintain higher soil inorganic N concentrations throughout the growing season compared with a single pulse of N fertilizer. Over the past 30 years, the mean annual precipitation was 1,045 mm, and the mean annual temperature was 10 °C at this site (Illinois climate network, 2017). During this time period, the average growing season (June–September) air temperature ranged 22–24 °C, and the mean April temperature averaged 11 °C (Illinois climate network, 2017).

#### 2.2. Study Design

To capture spatial and temporal variability in soil GHG emissions, net soil-atmosphere fluxes of N2O were measured 11 times from April 2017 through September 2017 from 65 sampling locations chosen to cover a range in soil series classifications and elevation found at the study site (Figure 1). These sampling dates included measurements the day after two large rain events in which over 30 mm of rain fell in a 24 hr period (on 1 May and 15 June) and after four smaller rain events (7 April, 17 April, 12 July, and 4 August). Surface ponding of soils was only observed following the 1 May rain event. LIDAR-derived topographic indices and soil series classifications were used to guide the selection of sampling locations. Sampling locations were chosen to span the elevation gradient in the field and to include areas that were identified as topographic depressions by the topographic fill model. Sampling locations were also chosen to span the different soil series present in the field, with the goal of covering spatial variability in soil properties relevant to soil drainage such as soil texture. There are three geographically associated soil series within the field that form a toposequence from upslope to depressional landform positions: Dana fine-silty soils, Flanagan silt loams, and Drummer silty clay loams. Soil series classifications were determined online using the Natural Resource and Conservation Service's Web Soil Survey tool (Soil Survey Staff, 2006).

## 2.3. Indices of Soil Drainage

LIDAR-derived topographic indices and magnetic susceptibility were used to identify areas likely to experience ponding following rain events. Elevation, latitude, and longitude of each sampling location was measured using a Trimble RTK GPS (Trimble, Sunnyvale, CA, USA) with accuracy of  $\pm 10$  cm. Localized depression index was calculated using a

hydraulic fill model based on a DTM derived from LIDAR data from the Illinois clearinghouse (Illinois State Geological Survey). This was done within ArcGIS v. 10.5 (ESRI, Redlands, CA, USA). Magnetic susceptibility was measured using a MS2 meter and MS2D loop attachment (Bartington Instruments Ltd, Oxon, England). Magnetic susceptibility is reported in dimensionless volume units (e.g.,  $\times 10^{-5}$ , SI units; Grimley et al., 2004).

#### 2.4. Soil Characteristics

To characterize soil properties that may be drivers of soil  $N_2O$  emissions, soil organic C concentration, pH, bulk density, and texture were also measured from a soil sample collected at each gas flux sampling location at the end of the growing season. A 5 cm diameter by 10 cm depth soil core was taken from each location using a stainless steel soil corer (AMS, Inc., American Falls, ID, USA). The soil samples were weighed and



air dried in the laboratory prior to analyses. Soil organic C (SOC) concentrations were measured on ground soil samples using a Vario Micro Cube elemental analyzer (Elementar, Hanau, Germany). Bulk density was calculated as the dry soil weight divided by the volume of the core; the mass of rocks and organic matter were subtracted from the soil mass. The pH of each sample was measured in a 1:1 ratio of dry soil to deionized  $H_2O$ . Soil texture was measured using a hydrometer method (Gee & Bauder, 1986). Inorganic N concentrations were not measured as they vary greatly over short timescales (Krichels et al., 2019; Sebilo et al., 2013).

#### 2.5. Soil Oxygen and Moisture Measurements

To determine how rain events affected surface and subsoil  $O_2$  concentrations throughout the field, we monitored bulk soil O<sub>2</sub> and volumetric soil moisture concentrations at 10 and 20 cm depths quasicontinuously in the field. Bulk O2 measurements measure O2 concentrations in soil pore space and may not detect anaerobic microsites within soil aggregates (Sexstone, 1985). We installed Apogee SO-110 oxygen sensors fitted with AO-001 diffusion heads (Apogee Instruments, Logan, UT, USA) and CS655 soil moisture probes (Campbell Scientific, Logan, UT) at four locations within the field on 4 April 2017. To minimize soil disturbance during installation of the  $O_2$  probes, we used a soil core to create a hole of a diameter matching that of the probes and of the desired sampling depth, gently inserted the probe into the hole, and repacked the hole with soil. To install the moisture probes, we dug a 30 cm deep hole adjacent to where the O2 sensors were installed, inserted the probes horizontally into the undisturbed soil at 10 and 20 cm depth, and repacked the hole with soil. Only one of the four sampling locations was outfitted with moisture probes on 4 April, and the rest were installed on 7 May. The locations were chosen to cover the east to west elevation gradient across the field (Figure 1a). Voltage from the sensors was measured every minute, and 30 min averages were recorded on a CR-100 datalogger (Campbell Scientific, Logan, UT). Voltage measurements were converted to relative percent  $O_2$ using a unique calibration factor for each probe empirically determined in the laboratory prior to field deployment. The probes were calibrated based on mV measurements at 0% O<sub>2</sub> (dinitrogen gas) and 20.95%  $O_2$  (ambient air) in sealed jars containing water to create a humid environment. An empirical function provided in the Apogee SO-110 owner's manual was used to correct soil O<sub>2</sub> concentrations for temperature in the diffusion head as measured by the SO-110 sensor and atmospheric pressure measured at a nearby weather station (Illinois climate network, 2017). Sensors were removed from the field from 18 May through 1 June for planting of G. max and from 29 June through 2 July for application of pesticides. Data are also missing from three of the sensors starting 22 July due to battery failures.

## 2.6. Field Trace Gas Measurements

Net soil-atmosphere N2O fluxes were measured manually using two-piece surface flux chambers. The chambers consisted of a 10 cm tall by 26 cm diameter vented acrylonitrile-butadiene-styrene chamber lid equipped with a rubber septum port and a vent placed on top of a PVC collar (Matson et al., 1990). The PVC collars were initially installed at each sampling location 1 week prior to the first sampling date on 7 April 2017. The collars were inserted 3-5 cm into the soil and were left in the field for the majority of the growing season. Collar height was measured on each sampling date, and the collars were inspected to ensure they were securely inserted into the soil. The collars had to be temporarily removed from 17 May through 1 June 2017 for planting of G. max and from 29 June through 2 July for the application of pesticides to the field. The collars were reinstalled within 10 cm of their original location as determined using a portable GPS (Trimble RTK). A 15 ml gas sample was collected from each chamber at 0, 10, 20, and 30 min after closing the lid. The gas samples were stored in 10 ml pre-evacuated glass vials sealed with thick rubber septa (Geo-Microbial Technologies, Inc., Ochelata, OK, USA) and aluminum crimps (Wheaton Industries Inc., Millville, NJ, USA). All flux measurements were performed between 10 a.m. and 3 p.m. to limit daily temperature changes; chambers were sampled in a different order each day to avoid systematic bias associated with sampling time. Additionally, air temperature within each chamber was measured to account for temperature effects on gas volume. After gas samples were collected, soil temperature and chamber temperature were measured using an Acorn Temp 5 meter (Oakton Instruments, Vernon Hills, IL, USA). All gas samples were analyzed on a Shimadzu GC-2014 (Shimadzu Scientific Instruments, Columbia, MD, USa) gas chromatograph (GC) equipped with an electron capture detector to measure concentrations of N<sub>2</sub>O. Net N<sub>2</sub>O fluxes were determined from the change in gas concentration over time using an iterative model that fits an exponential curve to the data (Matthias et al., 1978). Fluxes were recorded as zero when



there was no significant relationship between gas concentration and time (p > 0.05). Net fluxes from the field experiment are reported as mean  $\pm$  standard deviation.

#### 2.7. Laboratory Experimental Design

We carried out a lab experiment to test our hypothesis that cold temperatures inhibit  $N_2O$  emissions from inundated soils due to limited soil O<sub>2</sub> depletion by heterotrophic respiration. We sampled soils during the early and late growing season to determine if seasonal shifts in soil microbial community composition result in different response to temperature treatments. We also collected soils from a well-drained upslope portion of the field as well as from a poorly drained depressional portion of the field to determine if controls on soil N<sub>2</sub>O emissions differ based on topographic position. Intact soil cores were flooded and subjected to experimental treatments to manipulate soil  $NO_3^-$  concentrations and temperature in a full factorial design (n = 5). The NO<sub>3</sub><sup>-</sup> addition treatment was included to determine if soil N<sub>2</sub>O emissions were limited by soil inorganic N concentrations rather than temperature, as G. max fields are not fertilized. Half of the soil cores were amended with enough NO<sub>3</sub><sup>-</sup> to increase soil NO<sub>3</sub><sup>-</sup> concentrations by 3.25  $\mu$ g N-NO<sub>3</sub>·g<sup>-1</sup> dry soil, which corresponds to typical soil  $NO_3^-$  concentrations during peak growing season when Z. mays is planted (Krichels et al. 2019). After flooding the intact soil cores, soil temperature was manipulated by placing half of the cores in a cold room that averaged 2 °C over the course of the experiment. The rest of the cores were kept at room temperature (22 °C). Net N<sub>2</sub>O and CO<sub>2</sub> fluxes were measured from each core at 1, 2, and 5 days after flooding the cores. Soil  $O_2$  concentrations at 5 cm depth were measured from a subset of the cores immediately prior to each N2O and CO2 flux measurement.

We collected 20 intact soil cores (0–10 cm depth) from an upslope and a depressional portion of the field (40 cores total) in March and in September using a 5 cm diameter soil corer fitted with a plastic sleeve for each soil core (AMS, Inc., American Falls, ID, USA). The soils were allowed to equilibrate on the benchtop in the laboratory for 2 days before the experimental treatments were initiated. The soil cores were flooded by injecting 10 ml of deionized water into each core using a spinal tap needle to evenly distribute the water vertically throughout the core. This was done four times such that at least 40 ml of added deionized water completely saturated the soils and left minimal (< 0.5 cm) standing water above the soil surface. If 40 ml of water did not saturate the core, then an additional 5–10 ml was added. For the late season experiment, 60 ml of water was needed to ensure complete saturation. For the NO<sub>3</sub><sup>-</sup> addition treatment, a 1.02 mM KNO<sub>3</sub> solution in the initial 40 ml of DI used to flood the cores. The saturated soil cores were placed in quart-sized mason jars, which were left open but loosely covered with aluminum foil to keep the cores dark and to minimize water loss via evaporation. The intact cores were weighed daily to monitor water loss. All soils remained flooded for the duration of the experiment and no cores lost more than 1 ml of water over the course of the incubation.

To measure trace gas emissions from the soil cores, a 15 ml gas sample was collected from each jar immediately after sealing it with a lid equipped with butyl septum and again 4 hr later. Gas samples were stored in pre-evacuated 10 ml vials sealed with rubber septa and aluminum crimps (Wheaton Industries Inc., Millville, NJ, USA). Gas samples were analyzed on the GC as described earlier. Net trace gas fluxes were determined by calculating the linear change in jar headspace gas concentrations over the 4 hr incubation. A minimum detectable flux was calculated based on the standard deviation of three standard gas samples (1,001 ppm  $CO_2$  and 1.08 ppm  $N_2O$ ) run on the GC. The calculated detection limit for  $N_2O$  was 0.002 ppm·hr<sup>-1</sup> and for  $CO_2$  was 2.4 ppm·hr<sup>-1</sup>. Net fluxes below this limit were considered not detectable and estimated as zero.

To measure soil  $O_2$  concentrations, the intact soil cores were equipped with SP-PSt6-NAU Oxygen Sensor Spots (PreSens Precision Sensing, Regensburg, Germany). Each sensor spot was adhered to the inside of the soil core sleeve at 5 cm below the top of the core. These sensor spots measure gas and dissolved phase  $O_2$  concentrations and are read using a fiber optic cable that can communicate with the spot through the plastic core (Fibox4 trace, PreSens Precision Sensing, Regensburg, Germany). Due to a limited number of sensor spots, only soil cores that did not receive the  $NO_3^-$  addition were equipped with a sensor spot, with only three replicates per treatment randomly selected for  $O_2$  monitoring in the early growing season (12 cores total) and all five replicates per treatment monitored in the late growing season (20 cores total).



#### 2.8. Statistics

All statistical analyses were performed using R version 3.5.2 (R Core Team, 2018). We used linear mixed effects models to determine the best predictors of soil N<sub>2</sub>O flux on each sampling date. This was done using the lme function in the nlme package in R (Pinheiro et al., 2017). All N<sub>2</sub>O flux data were assessed for normality with a Shapiro-Wilk test conducted using the stats package in R (R Core Team, 2018); log transformations were applied to normalize the data. When flux values below 1 ng N-N<sub>2</sub>O cm<sup>-2</sup>·hr<sup>-1</sup> occurred from a chamber for a given sampling date, a constant of 1 was added to flux values prior to log transformation. Elevation, localized depression index, percent clay, magnetic susceptibility, soil organic C concentrations, and pH were included as fixed independent variables in the initial model. We then used Akaike information criterion (AIC) to select which model terms to include in each model using the AIC package in R (R core team, 2018). We tested for the occurrence of spatial autocorrelation by calculating Moran's I using the ape package in R (Paradis & Schliep, 2018). If significant spatial autocorrelation was detected, then we corrected for it by adding a correlation matrix based on chamber distance from one another as a random effect to the model. Separate models were run for each sampling date. On some days many chambers did not exhibit significant N2O emissions. However, we still ran our models on these days as hot spots can occur in part of the field even when other parts of the field have no detectable net soil-atmosphere N<sub>2</sub>O fluxes. We ran an additional model using data from all 11 sampling days to elucidate which temporal variables best explained N<sub>2</sub>O emissions from the entire field. For this model, soil temperature and total rainfall over the previous 48 hr were included as independent variables. Means and standard deviation are reported for elevation, localized depression index, percent clay, magnetic susceptibility, soil organic C concentrations, and pH. Relationships between these variables  $(R^2)$  were calculated using the lm function in R.

For the lab experiment, we ran mixed effects models to test for the effects of temperature,  $NO_3^-$ , and time since soil saturation on both  $N_2O$  emissions and  $CO_2$  emissions from the intact soil cores. Separate models were run for soils collected early versus late in the growing season, as well as for soils collected from upslope versus depressional areas of the field. In each model, incubation temperature,  $NO_3^-$  addition, and sampling day were included as fixed variables, and core ID was included as a random variable. Core ID was included as a random variable to account for repeated measurements of the same cores over the course of the experiment. The models were run using the lme function in the nlme package in R (Pinheiro et al., 2017). We then used the anova.lme function from the nlme package to determine which factors best predicted both  $CO_2$  and  $N_2O$  emissions. The same analyses were performed for soil  $O_2$  concentrations. For the soil  $O_2$  model,  $NO_3^-$  addition was not included as a factor as  $O_2$  concentrations were not measured from the cores that received  $NO_3^-$  addition.

#### 3. Results

#### 3.1. Soil Drainage Variables

The study field exhibited a microtopographic gradient, ranging from 223.6 m above sea level at the highest point to 222.6 m above sea level at the lowest (Figure 1). The topographic fill model showed that 30 of the 65 sampling locations were located in topographic depressions, which averaged 0.12 m in depth relative to the immediate surrounding terrain. None of these topographic depressions were located on Dana soils, which are the most elevated soil series in the toposequence at the site, but they were evenly distributed between Flanagan and Drummer soils (Figure 1b). Magnetic susceptibility averaged  $19.6 \times 10^{-5} \pm 12.1 \times 10^{-5}$  SI units ( $\pm$  standard deviation; Table 1) and was positively correlated with elevation ( $R^2 = 0.66$ , p < 0.001, n = 65; Table 2).

Soil clay content, SOC concentrations, and pH varied considerably within the field (Table 1). Soil clay content averaged  $24.9 \pm 4.82\%$  and ranged 15.2-34.3%. Soil organic C averaged  $2.30 \pm 0.22\%$  and ranged 1.88-2.72% throughout the field. Soil bulk density ranged  $1.09-1.50 \text{ g}\cdot\text{cm}^{-3}$ , while soil pH ranged 5.45-7.17. Soil organic carbon concentration and clay content were negatively correlated with elevation (SOC:  $R^2 = 0.38$ , p < 0.001, n = 65; clay:  $R^2 = 0.56$ , p < 0.001, n = 65), while soil pH exhibited a weak positive correlation with elevation ( $R^2 = 0.04$ , p < 0.05, n = 65, Table 2). Soil organic carbon concentration and clay content were also significantly positively correlated ( $R^2 = 0.42$ , p < 0.001, n = 65, Table 2).



Table 1

Bulk density (g dry soil  $cm^{-3}$ )

1.50

1.09

Mean, Standard Deviation, Minimum, and Maximum of Soil Properties Across 65 Sampling Locations Within the Study Field Standard deviation Maximum Soil property Mean Minimum 222.3 Elevation (masl) 222.6 0.30 223.6 Magnetic susceptibility 19.6 12.1 11.1 69.1 Soil organic C concentration (%) 2.30 0.22 1.88 2.72 Clay content (%) 24.9 4.82 15.2 34.3

0.08

#### 3.2. Rainfall, Soil O<sub>2</sub> Concentrations, and Soil Moisture

1.30

While cumulative precipitation between April and September 2017 was comparable to the previous five growing seasons (Figure 2), a greater proportion of this rainfall occurred early in the growing season. Between 1 April and 1 June 2017, 300 mm of rain fell, compared with an average of  $225 \pm 34$  mm during this same time in 2012 through 2016 (Figure 2). However, only 177 mm of rain fell between 1 June and 1 September 2017, compared with an average of  $337 \pm 51$  mm during this time frame in 2012 through 2016 (Figure 2).

In situ soil gas phase  $O_2$  concentrations in all four locations in the field decreased in response to large rain events in the early to middle growing season (Figure 3). After a 37.1 mm rain event on 1 May, soil  $O_2$ concentrations fell to 5–15% throughout the field at both 10 and 20 cm depths, while volumetric soil moisture reached 45%. After a 32 mm rain event on 14 June, soil  $O_2$  remained above 12% at 10 cm depth throughout the field and dropped to 0% at 20 cm depth in some parts of the field. Soil moisture at 10 and 20 cm depth increased by 5–10% following this rain event (Figures 3d and 3e). For the rest of the growing season (through the end of September), soil  $O_2$  concentrations at both soil depths remained close to atmospheric concentrations, despite moderate rain events and one large rain event (32 mm on 28 August) that occurred after 15 June (Figure 3a). During this time, soil moisture ranged between 25% and 38% throughout the field and increased by approximately 3% following the large rain event on 28 August.

#### 3.3. Field N<sub>2</sub>O Emissions

The highest N<sub>2</sub>O emissions were observed on 15 June, after a 32 mm rain event the previous day (Figures 4a and 4b). On this date, N<sub>2</sub>O emissions averaged  $66.5 \pm 42.6$  ng N-N<sub>2</sub>O·cm<sup>-2</sup>·hr<sup>-1</sup> (± standard deviation) from the 65 chambers. There was significant spatial autocorrelation in N<sub>2</sub>O flux (Moran's *I*, *p* < 0.05; Table 2). The mixed model explained 18% of the variation in N<sub>2</sub>O emissions on this date, with elevation, depression index, magnetic susceptibility, and pH as significant model terms (Table 3). Magnetic susceptibility was positively correlated with N<sub>2</sub>O emissions, while the rest of the model terms were negatively correlated with N<sub>2</sub>O emissions (Table 3).

The only other sampling date when all 65 chambers had significant N<sub>2</sub>O emissions was on 12 July, the day after a 24.4 mm rain event, when emissions averaged  $10.4 \pm 10.1$  ng N-N<sub>2</sub>O·cm<sup>-2</sup>·hr<sup>-1</sup>. There was no significant spatial autocorrelation in N<sub>2</sub>O emissions on this day (Moran's *I*, *p* = 0.09). The soil drainage model explained only 9% of the variation in N<sub>2</sub>O emissions, with magnetic susceptibility and pH as the only

two terms selected for the model. Magnetic susceptibility was positively correlated with  $N_2O$  emissions, and pH was negatively correlated with  $N_2O$  emissions (Table 3).

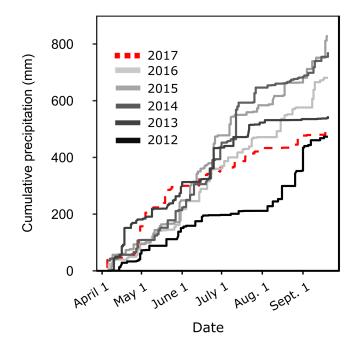
The models explained the most variation in N<sub>2</sub>O emissions on 10 August  $(R^2 = 0.29)$  and 17 September  $(R^2 = 0.28)$  when no rainfall had fallen in the previous 48 hr. On 10 August, N<sub>2</sub>O emissions averaged 2.24 ± 3.41 ng N-N<sub>2</sub>O·cm<sup>-2</sup>·hr<sup>-1</sup>, and 39 of the 65 sampling locations had significant N<sub>2</sub>O emissions (Figure 4). On this date, elevation and pH were both significant terms in the model (p < 0.05) and were negatively correlated with N<sub>2</sub>O emissions (Table 3). On 17 September, N<sub>2</sub>O emissions averaged 2.81 ± 3.09 ng N-N<sub>2</sub>O·cm<sup>-2</sup>·hr<sup>-1</sup>, and 49 of the 65 sampling locations had

Table 2	
<i>Coefficient of Determination</i> (R <sup>2</sup> ) <i>Between All Measured Soil Properties as</i>	
Determined Using Linear Models	

Soil property	Depression	Clay	MS	SOC	pН
Elevation Depression Clay MS SOC	0.16	0.56 0.11	0.66 0.07 0.22	0.38 0.04 0.42 0.10	<ul> <li>0.04</li> <li>0.06</li> <li>0.01</li> <li>0.03</li> <li>0.05</li> </ul>

*Note.* Bold values indicate significant correlation at p < 0.05.





**Figure 2.** Cumulative precipitation during the 2012–2017 growing seasons (1 April through 15 September). The red line represents the 2017 growing season when this study was conducted, and black through lighter gray lines represent 2012 through 2016.

significant N<sub>2</sub>O emissions (Figure 4). On this date, topographic depression index and SOC concentration were the only significant terms in the model (p < 0.05, Table 2), with both negatively correlated with N<sub>2</sub>O emissions. During the first five sampling dates (7 April through 7 June), N<sub>2</sub>O emissions averaged less than 1.0 ng N-N<sub>2</sub>O cm<sup>-2</sup>·hr<sup>-1</sup> on each day (Figure 4b), and 30–58 of the chambers did not exhibit detectable N<sub>2</sub>O fluxes.

Much of the temporal variation in soil N<sub>2</sub>O could be explained by the interaction between soil temperature and how much rain had fallen in the previous 48 hr ( $R^2 = 0.69$ , p < 0.001).

#### 3.4. Laboratory Experiment

Early in the growing season, there was a significant interactive effect of temperature and day of experiment on soil O<sub>2</sub> concentrations, CO<sub>2</sub> emissions, and N<sub>2</sub>O emissions from both upslope and depressional soils (Figure 5). Soil O<sub>2</sub> concentrations remained below 4% in soils incubated at room temperature (Figures 5a and 5d). In contrast, O<sub>2</sub> concentrations in soils incubated at 2 °C decreased slowly over the course of the incubation, from  $10.9 \pm 0.87\%$  to  $2.7 \pm 0.90\%$  in upslope soils (temperature \* day of experiment interaction:  $F_{2,10} = 4.27$ , p < 0.05; Figure 5a) and from  $13.6\% \pm 0.91\%$  to  $3.8 \pm 1.0$  in depressional soils (temperature \* day of experiment interaction:  $F_{2,10} = 18.2$ , p < 0.001; Figure 5d). Carbon dioxide emissions were consistently higher from soils incubated at 22 °C and increased over time in both temperature treatments (Figures 5b and 5e). This pattern was observed for upslope soils (temperature \* day of experiment interaction:  $F_{2,32} = 11.1$ , p < 0.001; Figure 5b) and depressional

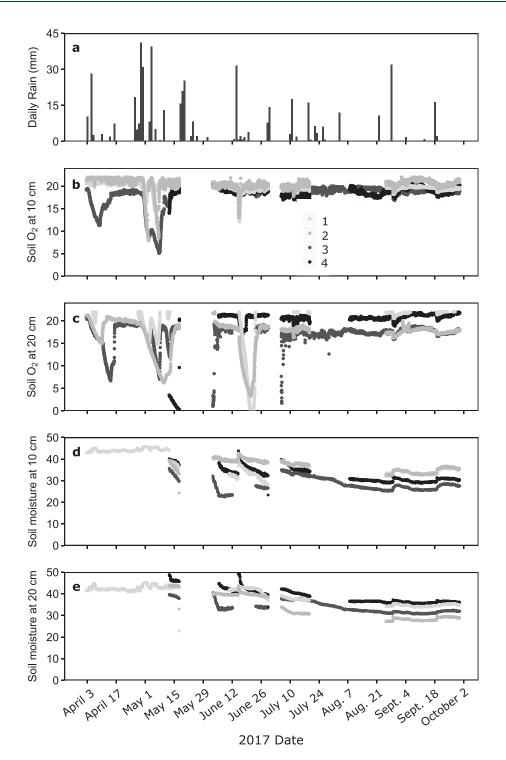
soils (temperature \* day of experiment interaction:  $F_{2,32} = 5.36$ , p = 0.01; Figure 5e). Nitrous oxide emissions from upslope soils were initially greater from soils incubated at 22 °C ( $0.93 \pm 0.16$  ng N·g<sup>-1</sup>·hr<sup>-1</sup>) compared with soils incubated at 2 °C ( $-0.01 \pm 0.01$  ng N·g<sup>-1</sup>·hr<sup>-1</sup>; Figure 5c). However, after remaining flooded for four days, this pattern reversed. In upslope soils, N<sub>2</sub>O emissions decreased to  $0.01 \pm 0.01$  ng N·g<sup>-1</sup>·hr<sup>-1</sup> at 22 °C and increased to  $0.86 \pm 0.58$  ng N·g<sup>-1</sup>·hr<sup>-1</sup> at 2 °C (temperature \* day of experiment interaction:  $F_{2,32} = 10.4$ , p < 0.001). Nitrous oxide emissions from depressional soils incubated at 22 °C also decreased over time, but N<sub>2</sub>O emissions from depressional soils incubated at 2 °C were more variable, and no obvious temporal pattern was observed (temperature \* day of experiment interaction:  $F_{2,32} = 3.98$ , p = 0.03; Figure 5f).

Late in the growing season, temperature effects and temporal patterns in soil O<sub>2</sub> concentrations and CO<sub>2</sub> emissions mimicked what was observed early in the growing season from both upslope and depressional soils (Figure 6). However, patterns in soil N<sub>2</sub>O emissions from upslope soils differed between sampling dates. Specifically, N<sub>2</sub>O emissions were highest from warm NO<sub>3</sub><sup>-</sup> amended upslope soils late in the growing season (temperature \* NO<sub>3</sub><sup>-</sup> addition interaction:  $F_{1,16} = 6.54$ , p = 0.02; Figure 6c), which was not exhibited in the early season. Soil N<sub>2</sub>O emissions from upslope soils incubated at 22 °C remained higher than N<sub>2</sub>O emissions from cold-treated soils for the entire late season experiment (Figure 6c). Additionally, the NO<sub>3</sub><sup>-</sup> amended upslope soils incubated at 22 °C had higher N<sub>2</sub>O emissions compared with the ambient NO<sub>3</sub><sup>-</sup> soils incubated at 22 °C throughout the late season experiment. In contrast, NO<sub>3</sub><sup>-</sup> addition did not affect N<sub>2</sub>O emissions from depressional soils during the late growing season ( $F_{1,16} = 2.3$ , p = 0.15). However, there was a significant interaction effect between temperature and day of experiment on N<sub>2</sub>O emissions from depressional soils late in the growing season ( $F_{2,32} = 15.4$ , p < 0.001; Figure 6f), as was observed from both upslope and depressional soils early in the early growing season (Figures 5c and 5f).

# 4. Discussion

Contrary to the static view of rainfall events as triggers of  $N_2O$  hot moments and topographic depressions as  $N_2O$  hot spots, our data show that hot moments of  $N_2O$  emissions do not always occur after large rain events and microtopographic depressions do not necessarily determine where hot spots of  $N_2O$  emissions develop. Pulses in soil  $N_2O$  emissions were only observed after one large rain event in June and not after rainfall in



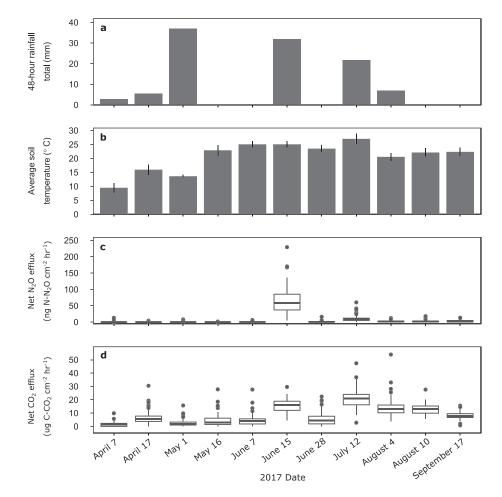


**Figure 3.** Daily precipitation (mm; a), soil  $O_2$  concentration (%) at 10 cm depth (b), soil  $O_2$  concentration at 20 cm depth (c), volumetric soil moisture (%) at 10 cm depth (d), and volumetric soil moisture at 20 cm depth (e). Different colors represent different sensor locations within the field as shown in Figure 1a. The numbers correspond to the locations shown in Figure 1a. Sensors were removed from the field from 18 May through 1 June for planting of *G. max* and from 29 June through 2 July for application of pesticides. Data are also missing from three of the sensors starting 22 July due to battery failures.

the early spring and late summer. Results from the lab experiment suggest that cold temperatures prevent the depletion of soil  $O_2$  concentrations by microbial respiration following large rainfall in the spring, delaying the onset of denitrification. Late in the growing season, high plant evapotranspiration rates may prevent large rain events from saturating soils and establishing anoxic conditions necessary for  $N_2O$ 

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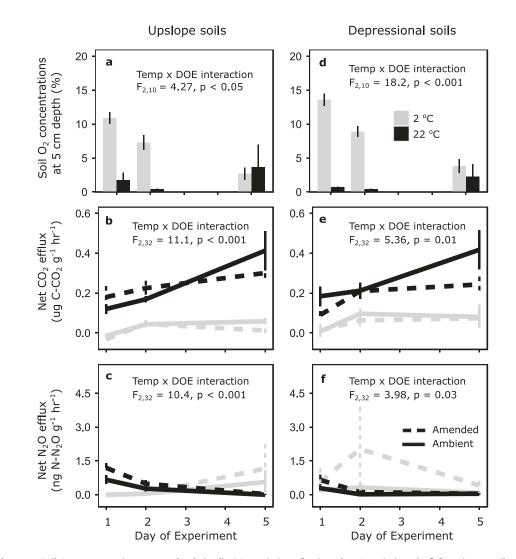
**Figure 4.** Total rainfall over the 48 hr prior to trace gas measurements (a), mean soil temperature  $\pm$  standard deviation (b), mean N<sub>2</sub>O emissions (c), and CO<sub>2</sub> emissions (d) as measured on 11 days over the course of the 2017 growing season. The line in each box in panels c and d represents the mean (n = 65), the upper and lower portions of each box correspond to the 25th and 75th percentiles, the whiskers extend to the upper and lower interquartile range, and dots represent outliers.

#### Table 3

Summary of Statistical Results From Soil Drainage Linear Mixed Effects Models of N2O Emissions on Each Sampling Date

Date	Elevation	Depression	Clay	MS	SOC	pН	$R^2$	Moran's I
7 April	-0.38	_	-0.06	0.02	0.63	_	0.22	0.11
17 April	_	_	_	_	—	_	0.00	0.39
1 May	0.14	_	_	-0.01	_	_	0.09	0.20
16 May	0.04	_	0.01	_	_	_	0.05	0.79
7 June	0.29	—	_	-0.02	—	-1.7	0.16	<0.01
15 June	-0.34	-0.36	_	0.02	_	-3.7	0.18	0.03
28 June	_	_	-0.04	_	_	_	0.08	0.83
12 July	_	_	_	-0.01	_	2.9	0.09	0.09
4 August	0.61	-0.04	_	_	1.89		0.19	< 0.01
10 August	-0.41	_	_	0.02	_	-5.1	0.29	< 0.01
17 September	—	-0.43	—	—	-0.87	—	0.28	<0.01

*Note.* The dependent variable in this model was  $\log(N_2O \text{ emissions} + 1)$ , and the independent variables were elevation, depression index, clay content, magnetic susceptibility (MS), soil organic C concentration (SOC), and pH. AIC selection criteria were used to select model terms; only the selected terms are reported for each model. The coefficient for each model term is presented; coefficients for statistically significant model terms (p < 0.05) are in bold. The total  $R^2$  for each model is also presented. When spatial autocorrelation was detected (Moran's I < 0.05), the model included a gaussian error term to account for this. The p value for Moran's I is also reported.



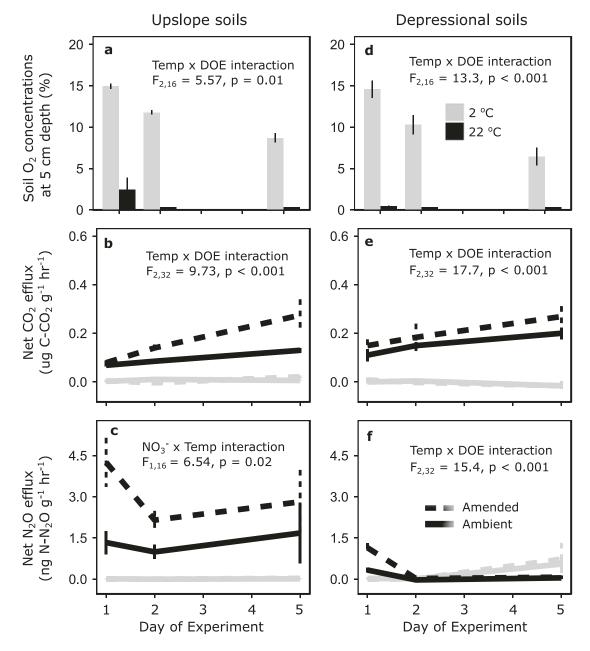
**Figure 5.** Soil O<sub>2</sub> concentration at 5 cm depth (a, d), CO<sub>2</sub> emissions (b, e), and N<sub>2</sub>O emissions (c, f) from intact soil cores for the lab incubation experiment conducted early in the growing season (n = 3 for a and d, n = 5 for b, c, e, and f). Error bars represent standard errors. Data for soils collected from an upslope portion of the field (a, b, c) and a depressional portion of the field (d, e, f) are shown separately. Gray lines correspond to soils incubated at 2 °C, while black lines correspond to soils incubated at 22 °C. Dashed lines represent soils amended with NO<sub>3</sub><sup>-</sup>, and solid lines represent ambient NO<sub>3</sub><sup>-</sup> concentrations.

production via denitrification. When significant  $N_2O$  emissions were measured, models including various proxies for microtopography did not explain much of the spatial variation in  $N_2O$  emissions. This may be a result of other controls over soil moisture, such as evapotranspiration, preventing spatial patterns in soil  $O_2$  concentrations. We also show that soil drainage legacy effects can develop over the course of the growing season and that this may obscure any spatial patterns in soil  $N_2O$  emissions associated with topographic variation at the field scale. Below, we explore possible mechanisms driving these patterns and what consequences they might have for predicting  $N_2O$  emissions in response to climate change.

We hypothesized that different indices of microtopography would best predict field-scale variation in soil  $N_2O$  emissions based on recent rainfall amounts but found that all indices we examined were weak predictors. While different topographic indices were significant model terms on each sampling date, no model explained more that 29% of the spatial variation in soil  $N_2O$  emissions on any given day (Table 3). This contrasts with prior observations that poorly drained topographic depressions consistently act as hot spots of soil  $N_2O$  emissions (Turner et al., 2016; Velthof et al., 1996; Yanai et al., 2003). However, these studies targeted measurements after fertilization that could have led to  $NO_3^-$  accumulation in depressions

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**Figure 6.** Soil  $O_2$  concentration at 5 cm depth (a, d),  $CO_2$  emissions (b, e), and  $N_2O$  emissions (c, f) from intact soil cores for the lab incubation experiment conducted late in the growing season (n = 5). Error bars represent standard errors. Data for soils collected from an upslope portion of the field (a, b, c) and a depressional portion of the field (d, e, f) are shown separately. Gray lines correspond to soils incubated at 2 °C, while black lines correspond to soils incubated at 22 °C. Dashed lines represent soils amended with  $NO_3^-$ , and solid lines represent ambient  $NO_3^-$  concentrations.

to create the N<sub>2</sub>O hot spots, whereas we intentionally conducted our study in unfertilized *G. max* field to isolate the effects of rainfall and topography on soil N<sub>2</sub>O emissions. The field-scale patterns in soil N<sub>2</sub>O emissions over the growing season suggest other variables not directly related to microtopography could be more important in explaining spatial variation in soil N<sub>2</sub>O emissions. For example, during peak growing season, high evapotranspiration rates from crops can draw large amounts of water out of soils (Sophocleous, 2002), helping retain oxic conditions that prevent denitrification from taking place (Knowles, 1982). As such, at peak plant biomass, high evapotranspiration rates may prevent depressional areas from experiencing hypoxic conditions necessary to drive spatial patterns in N<sub>2</sub>O emissions. Moreover, spatial variation in other soil properties such as microbial community composition (Philippot et al., 2009) or soil structure (Palta et al., 2016) may contribute to field-scale patterns in soil N<sub>2</sub>O

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emissions. This may explain why our models, which did not include these variables, only explained up to 18% of the variation in soil  $N_2O$  emissions after a large rain event in mid-June when emissions were the most variable across the field. Although our data did not support our hypothesis, they do suggest that the controls on spatial variation in soil  $N_2O$  emissions likely change over the course of the growing season and that topographic indices alone are insufficient to predict much of this variation.

The development of historical soil drainage legacy effects over the growing season can make it difficult to use microtopographic indices to predict the location of hot spots of N2O emissions. When upslope and depressional soils were incubated under the same inundated and warm conditions late in the growing season, the upslope soils exhibited higher N<sub>2</sub>O emissions compared with the depressional soils (Figure 6c). This laboratory observation of a soil drainage legacy effect is consistent with patterns in soil N<sub>2</sub>O emissions documented in a nearby field after large rain events that caused ponding in the middle to late growing season (Krichels et al., 2019). However, the soil drainage legacy effect was not manifested in the early growing season laboratory experiment (Figure 5c), suggesting that upslope and depressional soils have different controls over N<sub>2</sub>O dynamics that are established over the course of the growing season. For example, upslope and depressional soils can have distinct denitrifying microbial communities (Suriyavirun et al., 2019), and the active community composition may further diverge over the course of the growing season as a result of differences in soil  $O_2$ fluctuations (DeAngelis et al., 2010; Krichels et al., 2019) or plant-microbe interactions (Buyer et al., 2002; Mbuthia et al., 2015). Furthermore, differences in soil structure between upslope and depressional soils can alter the distribution of water and O<sub>2</sub> in soil pores (Algayer et al., 2014; Askaer et al., 2010; Sey et al., 2008), thus affecting soil denitrification rates (Krichels et al., 2019; Palta et al., 2016). Changes in soil structure can occur over the course of the growing season as a result of variable water fluctuations (De-Campos et al., 2009; Horn & Smucker, 2005; Six et al., 2004) and may be homogenized following annual tillage of agricultural fields (Six et al., 2004). As such, historical soils drainage legacy effects can be established over the course of the growing season, contributing to the dynamic nature of hot spots and hot moments of  $N_2O$  emissions.

Although precipitation is often associated with hot moments of soil N<sub>2</sub>O emissions (Dobbie & Smith, 2003; Li et al., 1992; Sexstone et al., 1985), we found that hot moments only occurred following large rain events during the middle of the growing season (Figure 4). Heavy rainfall stimulated the highest N<sub>2</sub>O emissions observed in this study during warm conditions in mid-June, but rain events of similar magnitude failed to stimulate hot moments in N<sub>2</sub>O emissions under cool conditions early in the growing season. In the laboratory, microbial respiration in cold soils was significantly lower than in warm soils and took multiple days to consume enough  $O_2$  to induce denitrification (Figures 5 and 6), supporting our hypothesis that low temperatures can indirectly inhibit denitrification. Temperature limitation of  $N_2O$  emissions occurred even when  $NO_3^-$  was added to soil incubations in the lab, suggesting that low N<sub>2</sub>O emissions observed in the field during the cool spring may not have been due to NO<sub>3</sub><sup>-</sup> limitation in unfertilized G. max fields. While the laboratory treatment was colder than spring soil temperatures in the field, cool soil temperatures in the field could still slow microbial respiration enough to delay the onset of hypoxic conditions for over 24 hr following rainfall, the timescale at which our field measurements were conducted. Assuming that rates of microbial respiration are twice as high at 12 °C compared with 2 °C ( $Q_{10} = 2$ ; Meyer et al., 2018) and that this doubles the rate of O<sub>2</sub> consumption compared with rates observed under cold conditions in the lab, it would take 47 hr for microbial respiration to establish anaerobic conditions at 12 °C. Higher O2 concentrations in cold incubated soils were not just a result of increased solubility of O<sub>2</sub> in cold water (Han & Bartels, 1996), as the low respiration rates under cold conditions still decreased dissolved O<sub>2</sub> concentrations over the 4 day incubation. While low temperatures can directly inhibit denitrification enzyme activity (Braker et al., 2010; Holtan-Hartwig et al., 2002), N<sub>2</sub>O emissions from soils increased after 4 days of flooding under cold conditions. This increase was concomitant with a decline in soil  $O_2$  concentrations, suggesting that the presence of  $O_2$  rather than direct temperature limitation on denitrifier activity suppressed N<sub>2</sub>O emissions under cold conditions. Ponding of depressional areas typically only lasts 1-2 days following large rainfall (Krichels et al., 2019), which may not be long enough to stimulate denitrification under cold conditions. An alternative explanation to N2O hot moments occurring only in the summer is that interludes between rain events were longer (up to 18 days) in the summer than in the spring, potentially leading to the Birch effect driving trace gas emissions by releasing labile C upon wetting of the soil (Birch, 1958). However, the mesic soils in our study remained above 20% volumetric soil moisture (Figures 3d and 3e) and therefore did not dry to the same extent observed during the months-long dry season in Mediterranean



ecosystems where the Birch effect plays an important role (Austin et al., 2004). Taken together, these results suggest that the development of  $N_2O$  hot spots following spring rainfall can be delayed if cool temperatures constrain  $O_2$  consumption in soil pore water.

In conclusion, we show that the controls on the location and timing of hot spots and hot moments of N<sub>2</sub>O emissions are dynamic over the course of the growing season. Microtopographic depressions did not consistently act as hot spots of N<sub>2</sub>O emissions, and large rain events only triggered hot moments of N<sub>2</sub>O emissions under warm conditions during peak growing season. Seasonal changes in temperature, crop growth, and soil microbial community composition may mediate the effects of topography and rainfall on soil N<sub>2</sub>O emissions, leading to dynamic controls on the formation of N<sub>2</sub>O hot spots and hot moments. Global climate models predict that a greater proportion of cumulative annual rainfall will occur in spring instead of summer months, resulting in wetter springs and drier summers (USGCRP 2009; Min et al., 2011). Changes in soil greenhouse gas emissions in response to changing precipitation regimes have the potential to feedback on climate change. Our findings suggest that cool temperatures may constrain  $N_2O$ hot moments in response to spring precipitation and that shallow topographic depressions may not harbor N<sub>2</sub>O hot spots during drier summer months. This suggests that increased frequency and magnitude of precipitation events may not necessarily increase soil N<sub>2</sub>O emissions to have a positive feedback on climate change. Moving beyond a static view of depressions and large rain events as triggers for N<sub>2</sub>O hot spots and hot moments could allow us to better predict ecosystem scale changes in soil N<sub>2</sub>O emissions resulting from changing precipitation regimes.

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