X-ray computed tomography to predict soil N\textsubscript{2}O production via bacterial denitrification and N\textsubscript{2}O emission in contrasting bioenergy cropping systems

Alexandra N. Kravchenko\textsuperscript{1,2} | Andrey K. Guber\textsuperscript{1} | Michelle Y. Quigley\textsuperscript{1}
John Koestel\textsuperscript{3} | Hasand Gandhi\textsuperscript{4} | Nathaniel E. Ostrom\textsuperscript{2,4}

\textsuperscript{1}Department of Soil, Plant and Microbial Sciences, Michigan State University, East Lansing, Michigan
\textsuperscript{2}DOE Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, Michigan
\textsuperscript{3}Swedish University of Agricultural Sciences, Uppsala, Sweden
\textsuperscript{4}Department of Integrative Biology, Michigan State University, East Lansing, Michigan

Abstract
While renewable biofuels can reduce negative effects of fossil fuel energy consumption, the magnitude of their benefits depends on the magnitude of N\textsubscript{2}O emissions. High variability of N\textsubscript{2}O emissions overpowers efforts to curb uncertainties in estimating N\textsubscript{2}O fluxes from biofuel systems. In this study, we explored (a) N\textsubscript{2}O production via bacterial denitrification and (b) N\textsubscript{2}O emissions from soils under several contrasting bioenergy cropping systems, with specific focus on explaining N\textsubscript{2}O variations by accounting for soil pore characteristics. Intact soil samples were collected after 9 years of implementing five biofuel systems: continuous corn with and without winter cover crop, monoculture switchgrass, poplars, and early-successional vegetation. After incubation, N\textsubscript{2}O emissions were measured and bacterial denitrification was determined based on the site-preference method. Soil pore characteristics were quantified using X-ray computed microtomography. Three bioenergy systems with low plant diversity, that is, corn and switchgrass systems, had low porosities, low organic carbon contents, and large volumes of poorly aerated soil. In these systems, greater volumes of poorly aerated soil were associated with greater bacterial denitrification, which in turn was associated with greater N\textsubscript{2}O emissions ($R^2 = 0.52$, $p < 0.05$). However, the two systems with high plant diversity, that is, poplars and early-successional vegetation, over the 9 years of implementation had developed higher porosities and organic carbon contents. In these systems, volumes of poorly aerated soil were positively associated with N\textsubscript{2}O emissions without a concomitant increase in bacterial denitrification. Our results suggest that changes in soil pore architecture generated by long-term implementation of contrasting bioenergy systems may affect the pathways of N\textsubscript{2}O production, thus, change associations between N\textsubscript{2}O emissions and other soil properties. Plant diversity appears as one of the factors determining which microscale soil characteristics will influence the amounts of N\textsubscript{2}O emitted into the atmosphere and, thus, which can be used as effective empirical predictors.
1 | INTRODUCTION

Renewable biofuels provide a unique opportunity for reducing the negative effects of fossil fuel energy consumption (Qin, Zhuang, & Zhu, 2015). However, the benefits of biofuel cropping systems may be offset by their contribution to greenhouse gas emissions, in particular, N2O (Johnson & Barbour, 2016; Oates et al., 2016; Qin et al., 2015; Walter, Don, & Flessa, 2015; Wightman, Duxbury, & Woodbury, 2015). Despite major efforts by scientific community to curb uncertainties in estimations of N2O (Bahl et al., 2013). Due to soil heterogeneity, the enormous microscale variability of the soil characteristics that influence these processes overpowers prediction efforts.

The majority of N2O production in soils is driven by biologically mediated nitrogen transformations, among which, nitrification and denitrification are generally regarded as the most influential processes in majority of terrestrial ecosystems (Barnard, Leadley, & Hungate, 2005; Fowler et al., 2009). Out of the two, denitrification is frequently found to be highly spatially variable and more difficult to predict, while often the dominant source of soil N2O production and emission are well understood (Butterbach-Bahl, Baggs, Dannenmann, Kiese, & Kessler, 2012; Huang, Grace, Mengersen, & Weier, 2011), which negatively affects the accuracy in estimating the benefits of biofuel systems. Even though the main processes behind soil N2O production and emission are well understood (Butterbach-Bahl et al., 2013), the enormous microscale variability of the soil characteristics that influence these processes overpowers prediction efforts.

15N and 18O vary or isotopically fractionate (Frame & Casciotti, 2010; Haslun, Ostrom, Hegg, & Ostrom, 2018; Sutka et al., 2006). Specifically, pure culture studies demonstrate that δ15N values of 33‰–37‰ and −10 to 0‰, respectively, can be used to indicate N2O production from hydroxylamine oxidation/denitrification and bacterial denitrification (Sutka et al., 2006; Sutka, Ostrom, Ostrom, Gandhi, & Breznak, 2004). Based on these values, the proportion of N2O derived from bacterial denitrification can be determined from the δ18O value of soil-derived N2O (Ostrom & Ostrom, 2011). Reduction of N2O during denitrification has the potential to alter δ15N; however, the magnitude of this effect is small (Jinuntuya-Nortman, Sutka, Ostrom, Gandhi, & Ostrom, 2008; Ostrom et al., 2007). Opdyke, Ostrom, and Ostrom, (2009 estimated that if 10% of produced N2O was reduced, the shift in δ15N would only be 0.7‰ and, further, would result in an underestimate of the importance of production from bacterial denitrification. The potential for abiotic production of N2O in soils is increasingly being recognized and may be particularly important in Fe-rich soils and in the presence of nitrite (Zhu-Barker, Cavazos, Ostrom, Horwath, & Glass, 2015). Although potentially activities of soil microorganisms consuming O2. Emission of N2O out of the soil also depends on the severity of O2 depletion, which can facilitate N2O reduction to N2, and on the presence of N2O escape routes, both governed by gas diffusion (Balaine et al., 2013; Ball, 2013; Mutegi, Munkholm, Petersen, Hansen, & Petersen, 2010). Further complicating matters are patterns of soil wetting/drying, resulting in differences in water distributions in the pore space for the same water contents, thus affecting gas diffusion rates in different parts of pore space and causing trapping of N2O in air-disconnected pores (Guo, Drury, Yang, Reynolds, & Fan, 2014; Rabot, Henault, & Cousin, 2014, 2016). Yet another complication is added by influences on N2O production from highly spatially variable N and C sources, such as decomposing plant residues (Kravchenko et al., 2017; Parkin, 1987).

N2O production in soils is a consequence of the activity of nitrifying and denitrifying microorganisms and potentially abiotic processes. Definitive resolution of production pathways remains challenging; however, isotopic site-preference (SP) analysis can provide considerable insight. SP is defined as the difference in δ15N between the central and outer N atoms in N2O and has been shown to be constant during microbial production even though δ15N and δ18O vary or isotopically fractionate (Frame & Casciotti, 2010; Haslun, Ostrom, Hegg, & Ostrom, 2018; Sutka et al., 2006). Specifically, pure culture studies demonstrate that SP values of 33‰–37‰ and −10 to 0‰, respectively, can be used to indicate N2O production from hydroxylamine oxidation/denitrification and bacterial denitrification (Sutka et al., 2006; Sutka, Ostrom, Ostrom, Gandhi, & Breznak, 2004). Based on these values, the proportion of N2O derived from bacterial denitrification can be determined from the SP value of soil-derived N2O (Ostrom & Ostrom, 2011). Reduction of N2O during denitrification has the potential to alter SP; however, the magnitude of this effect is small (Jinuntuya-Nortman, Sutka, Ostrom, Gandhi, & Ostrom, 2008; Ostrom et al., 2007). Opdyke, Ostrom, and Ostrom, (2009 estimated that if 10% of produced N2O was reduced, the shift in SP would only be 0.7‰ and, further, would result in an underestimate of the importance of production from bacterial denitrification. The potential for abiotic production of N2O in soils is increasingly being recognized and may be particularly important in Fe-rich soils and in the presence of nitrite (Zhu-Barker, Cavazos, Ostrom, Horwath, & Glass, 2015). Although potentially

KEYWORDS
bacterial denitrification, computed microtomography, particulate organic matter, plant diversity, site-preference analysis, soil pore size distributions
variable (Buchwald, Grabb, Hansel, & Wankel, 2016), the $S_p$ of $N_2O$ produced by abiotic processes is generally in the range of $26\%–35\%$ (Grabb, Buchwald, Hansel, & Wankel, 2017; Heil et al., 2014; Heil, Liu, Vereecken, & Bruggemann, 2015) and thus similar to the range expected for production via hydroxylamine oxidation and fungal denitrification. Bacterial denitrification largely stands alone with $S_p$ values primarily in the range of $0\%–10\%$ which provides a basis to constrain this process relative to other production pathways (Ostrom & Ostrom, 2011).

Most physical and biological processes that contribute to $N_2O$ emissions vary in soil at scales of microns to millimeters (Kravchenko, Negassa, Guber, & Rivers, 2015; Nunan, Wu, Young, Crawford, & Ritz, 2003), and their variations may not always be well captured by bulk soil characteristics, for example, soil water content, measured on decimeter-scale soil samples (Ball, 2013). Size distribution, connectivity, tortuosity, and saturation of soil pores govern air and water fluxes in soil and, thus, influence $N_2O$ production and $N_2O$ emission from soil at microscales (Ball, 2013; Schurgers, Dorsch, Bakken, Leffelaar, & Hau gen, 2006). However, these microscale characteristics are extremely difficult to quantify. One way to do this is by using X-ray computed microtomography ($\mu$CT) that enables visualization of soil pore geometry (Vogel, Weller, & Schluter, 2010; Wang, Kravchenko, Smucker, Liang, & Rivers, 2012). Moreover, it enables detection of plant roots and large fragments of organic material, that is, particulate organic matter (POM) (Kravchenko, Negassa, Guber, & Schmidt, 2014; Mooney & Morris, 2008). X-ray $\mu$CT images of intact soil samples allow analysis of these properties in situ and quantification of their influence on $N_2O$ emissions (Mangalassery et al., 2014; Mangalassery, Sjogersten, Sparkes, Sturrock, & Mooney, 2013; Porre, Groenigen, Deyn, Goede, & Lubbers, 2016; Rabot, Lacoste, Henault, & Cousin, 2015).

The goal of this study was to assess the contribution of soil pore characteristics and POM to $N_2O$ production via bacterial denitrification and to $N_2O$ emissions from soils under several contrasting bioenergy cropping systems. We measured pore characteristics by $\mu$CT scanning and production of $N_2O$ from bacterial denitrification using $S_p$ analysis. The studied systems are continuous corn with and without winter cover crop, monoculture switchgrass, poplars, and early-successional vegetation. We hypothesize that long-term growth (9 years) of different plant species affects soil C and N levels as well as the presence, connectivity, and size distributions of soil pores. These characteristics, in turn, will influence C and N availability to microorganisms and will govern the ability of soil to retain water and to enable gas exchange, and thus will both directly and indirectly affect the $N_2O$ production from denitrification and the emissions of $N_2O$ overall.

Our first objective was to study presence, connectivity, and size distribution of soil pores with radii $>30 \mu m$, under the assumption that their absence leads to anoxic conditions, while presence contributes to $O_2$ and $N_2O$ in- and outflows. The second objective was to explore the role of POM on $N_2O$ production and emission. We hypothesized that greater presence of POM surrounded by soil with prevalence of large pores will lead to greater $N_2O$ emissions. POM is a source of C and N, and its active decomposition can lead to local anaerobic conditions—both factors favoring $N_2O$ production—while the presence of large pores in its vicinity would enhance air diffusion and therefore cause larger emissions of produced $N_2O$.

In addition, we explored the role of water distribution patterns in $N_2O$ production and emission. Soil moisture and matric potential are the characteristics that define which soil pores and to which extent are filled with water. Due to the hysteresis of soil water retention, to which extent soil pores are filled with water at any moisture content and matric potential level depends on the history of soil wetting/drying. Generally, at the same matric potential, soil water content is higher, when an initially saturated soil is drained, as compared with a dry soil after rewetting. Because of hysteresis in soil water retention, the spatial distributions of water within the pore space vary. On the one hand, drying of the wet soil forms pockets of trapped water, which reduces gas diffusion and enhances local anaerobic conditions. On the other hand, rewetting of dry soil results in trapping air in dead-end pores, which also affects the gas diffusion and local anaerobic conditions in soils. To which extent the hysteresis of soil water retention affects $N_2O$ production and $N_2O$ emissions from soil is still unknown. We hypothesized that even though soil subjected to the wetting-up mode will have the same soil moisture or the same matric potential level as the soil subjected to the drying-down mode, it will still have lower $N_2O$ production via denitrification and lower $N_2O$ emissions.

## 2 MATERIALS AND METHODS

### 2.1 Experimental site and studied bioenergy systems

The experimental site is Great Lakes Bioenergy Center's Biofuel Cropping System Experiment located at Kellogg Biological Station, Michigan, USA, on well-drained Alfisol of Oshtemo and Kalamazoo series (mesic Typic Hapludalf; Robertson & Hamilton, 2015). The field experiment has been set up in 2008 as a randomized complete block design with five replications and with bioenergy systems assigned at random to 0.12 ha experimental plots within each replicated block. The five studied systems are two agronomic treatments: continuous corn (Zea mays L.) and continuous...
corn with winter cover crop of cereal rye (Secale cereale L.), a monoculture switchgrass (Panicum virgatum L.), a hybrid poplar (Populus nigra × P. maximowiczii “NM6”) with herbaceous understory (Sprunger & Robertson, 2018), and an early-successional community abandoned from agriculture in 2008. The experimental site was plowed prior to establishment, and no further plowing took place in any of the systems. The two continuous corn systems were managed as no-till. In the two corn systems, 168 kg N/ha of nitrogen fertilizer is applied annually, with 32 kg N/ha at planting and 136 kg N/ha as side-dress. Annually, 56 kg N/ha is applied to switchgrass and early-successional systems. The poplar system received a single application of 155 kg N/ha in 2010 and no annual applications of N. It should be noted that while, in general, these systems can be grown for several practical purposes, in this study, during the entire field experiment, all the systems were managed exclusively for bioenergy production. Thus, we refer to them as bioenergy systems. Detailed descriptions of the agronomic protocols, biomass harvest, and aboveground residue management are provided by Sanford et al. (2016) and Sprunger, Oates, Jackson, and Robertson (2017).

2.2 | Soil sampling

In this study, we collected soil samples only from four of the five replicated blocks of the field experiment. Intact soil cores (5 cm in diameter, 5 cm in height) were collected from four experimental plots of each cropping system, with 2–3 locations sampled within each plot, resulting in a total of 10 soil cores per cropping system. For each system, two cores were used for hysteresis measurements, and eight cores, that is, two cores from each experimental plot, were used in the incubation experiment. The cores were taken from depth of 5–10 cm in February of 2017, weighted, wrapped in an aluminum foil, and stored at 4°C.

2.3 | Water retention hysteresis

In order to determine hysteresis in water retention of the studied treatments, water retention was measured using a modified evaporation method in two soil cores per cropping system (Wind, 1968). Round bottom ceramic cups (0.95 cm OD, 2.858 cm length, type 0652X07-B01M3, Soilmoisture Equipment Corp., Santa Barbara, CA) connected via pressure transducers (PX26-030DV, Omega Engineering, Inc., Stamford, CT) to a panel meter (DP25B, Omega Engineering, Inc., Stamford, CT) were installed into each core to record pressure head in the soil. Soil cores were, first, capillary saturated by keeping them for 48 hr on water-saturated coarse sand and then gradually fully saturated by increasing the water level around the cores. Saturated cores were weighed and subject to air-drying from the upper surface of the cores. The changes in the pressure head were recorded hourly. Once the change in the pressure head value approached 4–5 kPa, the cores were covered with a plastic cap to stop evaporation and kept for 24 hr to let water pressure equilibrate with the water content in the soil core. Then, soil cores were weighed, and the evaporation procedure was repeated. As a result, we obtained 15–20 approximately evenly spaced data points for the soil water retention curve. The measurements of the drainage curve of the water retention were stopped at pressure head \( h = -70 \) kPa.

The saturation limb of the soil water retention was measured in the same samples by incremental addition of water to the soil, followed by recording the pressure heads and weights of cores upon equilibrating pressure heads. The experiment was conducted until full soil saturation, that is, zero pressure head registered by the tensiometers. Then, soil water content was measured gravimetrically. The changes in the core weights were used for the soil moisture calculations at each pressure head.

2.4 | Incubation experiments

The remaining soil cores were split into three groups to study the effect of hysteresis in water retention on \( \text{N}_2\text{O} \) emission rates from the soil. The first group consisted of the cores that after full saturation were brought to \(-10 \) kPa pressure using a 5 bar pressure plate extractor (Soilmoisture Equipment Corp., Santa Barbara, CA) and are referred to as drying treatment (Dry). The second and third groups included soil cores that were, first, subject to drainage at \(-70 \) kPa and then rewetting, and are referred to as treatments rewetted to same pressure (WetPr) and to the same water content (WetWC). The WetPr treatment was rewetted to \(-10 \) kPa pressure head, while the WetWC treatment received water in the same amounts that were lost during the core drainage from \(-10 \) to \(-70 \) kPa. Therefore, presumably Dry and WetPr had the same pressure heads, but different water contents, while the Dry and WetWC treatments had similar water contents, but different pressure heads (Supporting information Figure S1). The number of replicated cores was 3 in the WetWC and Dry treatments and 2 in the WetPr treatment for each cropping system. Soil preparation for the incubation experiment was conducted in a cold room at 4°C to reduce the microbial activity and took from 7 to 10 days, depending on the time needed for saturation.

After soil cores were brought to their respective hysteresis conditions and weighed, a 1 cm layer was nondestructively cut from each core and used for water content determination. The cores were then placed into 16 oz Mason jars with two sealed rubber stoppers in the jar caps. A vial with 10 ml of water was placed into each jar to maintain high humidity in the jar and to prevent water evaporation from the soil. The seal in the lids was tested.
using compressed air to prevent N₂O losses from the jars during incubation. N₂O content and S_p were measured after 72 hr incubation in the dark at temperature of 20°C, as described below. The soil samples were weighed to assure the absence of water content losses and then wrapped and stored in the cold room till X-ray µCT analysis.

2.5 | N₂O and S_p analysis

The relative importance of bacterial denitrification (including nitrifier denitrification) to total N₂O production was determined as described by Ostrom et al. (Ostrom et al., 2010). Specifically, pure culture studies demonstrate that S_p values of 33 to 37 and −10 to 0 ‰, respectively, indicate N₂O production from hydroxylamine oxidation + fungal denitrification and bacterial denitrification (Sutka et al., 2006). Based on these values, the proportion of N₂O derived from bacterial denitrification can be determined from the S_p value of soil-derived N₂O (Ostrom & Ostrom, 2011). We provide two estimates of the proportion of N₂O derived from bacterial denitrification: conservative and non-conservative based on endmember S_p values of −10‰ and 37‰ and 0‰ and 33‰, respectively (Kravchenko et al., 2017). The N₂O obtained from the soil cores was analyzed using a Trace Gas System (Elementar) interfaced to an Elementar Isoprime 100 mass spectrometer for determination of bulk δ¹⁵N, δ¹⁸O, and S_p (Sutka et al., 2006). Within the Trace Gas System, water and CO₂ are removed using chemical scrubbers (magnesium perchlorate and Carbosorb, respectively), and N₂O is chromatographically separated from the residual CO₂ on a Porapak Q column that is interfaced to the mass spectrometer (Sutka et al., 2006). We applied corrections for the contribution of ¹⁷O to masses 31 and 45 and for a small degree of rearrangement of ¹⁵N between the α and β positions within the ion source (Toyoda & Yoshida, 1999). The concentration of N₂O is determined from the peak area of the m/z 44 trace during isotopic analysis with a reproducibility (1 SD) of 3% or better (Ostrom et al., 2010). The internal laboratory N₂O isotope standards were calibrated by analysis of USGS51 and USGS52 pure gas standards that have reported δ¹⁵N, δ¹⁸O, δ¹⁵N*, δ¹⁵N^µ, and S_p values of 1.32, 41.23, 0.48, 2.15, and −1.67 and 0.44, 40.64, 13.52, −12.64, and 25.15‰, respectively (Ostrom et al., 2018).

2.6 | X-ray scanning

After incubation experiments, the cores were subjected to X-ray scanning using a GE Phoenix v|tome|x at the Institute of Soil and Environment at the Swedish University of Agricultural Sciences in Uppsala. The X-ray scanner was equipped with a 240 kV tube, a tungsten target, and a 16° flat panel detector with 2,048 × 2,048 detector crystals (GE 1600). Each 3D X-ray µCT image was reconstructed from 2,000 projections acquired at a tube voltage of 130 kV and an electron flux of 200 µA. Each projection was obtained from the average of three consecutive radiographs recorded at the individual projection angles. The exposure time per radiograph was set to 200 ms. No optical filters were used during the image acquisition. 3D µCT X-ray images were reconstructed from the projections using the GE software datosix. Each image had a resolution 29 µm in all directions.

2.7 | Image analysis

The X-ray µCT images were processed in ImageJ/Fiji software (Schindelin et al., 2012). A 2 voxel radius 3D median filter was applied to all images to remove random noise. A region of interest with a diameter and height of 4.5 cm was selected from the central portion of each µCT for the following analyses to avoid sampling artifacts close to the column walls.

For POM determination, we, first, visually identified a subset of 5–12 POM fragments from each core based on the grayscale value, size, and shape characteristics. The range of gray scale values was obtained from the central portion of each fragment. The average of the minimum and maximum gray scale values were then used as a threshold for initial POM identification. Then, a set of erosion/dilation steps was applied to eliminate boundary artefacts, followed by 3D Gaussian filter, and subsequent segmentation. Particle Analyser plugin (BoneJ) was then used to select only POM fragments with volume exceeding 0.016 mm³.

Visible gravel/stone fragments >2 mm in size were delineated on the images using single threshold segmentation. Due to their higher attenuation coefficients, most stones are much brighter, that is, have higher grayscale values, than the other soil components, and thus are easy to segment. After thresholding, the >2 mm fraction was identified using Particle Analyser plugin with particle size >8 mm³.

The X-ray resolvable soil pore network was obtained by first removing the areas delineated as POM and gravel from the X-ray images. The resulting images exhibited histograms in which gray scale values associated with air-filled pores were easily distinguished from ones pertaining to the soil matrix by using the minimum method. Pore size distributions with spatial locations of different pore radii were obtained using the maximal inscribable sphere method of Pore size distribution tool of Xlib plugin for ImageJ (Münch, 2008).

Given the scanning resolution of our µCT analysis, we could identify pores with radii >30 µm. In the subsequent description and discussion of the results, we interchangeably refer to such pores as either >30 µm pores or as visible pores.
To assess the size of the soil matrix that was not in close contact with >30 µm pores, we delineated a 180 µm wide zone around each µCT visible pore. It was assumed that due to proximity to large water-free pores, the soil within these zones has better aeration, than the remaining soil matrix. While choosing 180 µm for this delineation, we explored the patterns of changes in soil volume for a 30–810 µm range of distances from the pores (Supporting information Figure S1). The studied systems differed from each other the most in a 180–630 µm range, and 180 µm was consistent with spatial correlations reported for biological characteristics and soil C at microscales (Nunan et al., 2003; Quigley, Rivers, & Kravchenko, 2018); however, its choice is somewhat arbitrary in terms of gas diffusion. Delineation of 180 µm zone was conducted as a series of 3D dilations using 3D Dilate tool of ImageJ. The difference between the number of soil matrix voxels and the number of voxels belonging to the within the 180 µm zone was used in further data analyses as an indicator of the size of the volume of poorly aerated soil, and is referred to as Vol-180.

In order to assess POM’s aeration status, we overlaid the 3D POM images with the images of µCT visible soil pores and identified the POM fragments that were or were not connected to the soil surface by the µCT visible pores. These two groups of POM fragments are referred to, respectively, as connected and unconnected POM.

### 2.8 Statistical analysis

Comparisons among the bioenergy systems and hysteresis treatments in terms of the studied soil and N₂O measurements were conducted using a statistical model with the fixed effects of bioenergy system, hysteresis treatment, and their interaction and with the random effects of the two blocking factors involved in the experiment, that is, the experimental field blocks and the laboratory-processed blocks. Normality of the residuals and homogeneity of variances were checked for each studied variable. In case of marked deviations from normality, the data were log-transformed, for example, cumulative amount of emitted N₂O, while in case of variance heterogeneity, unequal variance analysis was performed (Milliken & Johnson, 2001). When inherent characteristics of the soil cores were correlated with N₂O measurements, for example, with WC during saturation, we conducted analysis of covariance (ANCOVA) and compared the effects of systems and hysteresis treatments after accounting for variations in these additional soil core characteristics (Milliken & Johnson, 2009). The analyses were conducted using PROC MIXED procedure of SAS (SAS Inc, 9.4).

Relationships among the studied continuous variables were first explored using correlation analyses conducted using the PROC CORR procedure of SAS and then were followed by fitting the relationships that were nonlinear with polynomial regression models using either PROC REG or PROC MIXED procedures. Comparisons among the studied systems in terms of parameters of the regression equations relating N₂O and soil variables, for example, regression slopes, were conducted using ANCOVA approach in PROC MIXED (Milliken & Johnson, 2009). Path analysis for examining influences on N₂O emissions was conducted as described in Wuensch (2016).

### 3 RESULTS

#### 3.1 Soil characteristics

The soils of the studied bioenergy systems differed in a number of characteristics (Table 1). The two systems with high diversity of plant communities, that is, poplars and early-successional system, had significantly larger soil organic C and N contents than the two no-till corn-based systems. Poplar and early-successional systems had markedly higher total porosity and higher presence of visible pores. However, in terms of the presence of pores <30 µm the studied systems did not differ from each other.

The three systems with perennial vegetation, that is, switchgrass, poplars, and early-successional community, tended to have higher levels of POM than the annual corn systems, and continuous corn had significantly lower POM than the rest of the systems (p < 0.1). The volume of POM connected to the atmosphere by >30 µm pores in poplar and early-successional systems tended to be higher than that in corn with cover crops and switchgrass and exceeded that in the continuous corn.

The Vol-180, that is, the volume of poorly aerated soil, was the lowest in soils from poplar, followed by early-successional system, with the two not significantly different from each other, and was higher in the two corn systems and the switchgrass. At the time of field sampling, the systems did not differ in their water content levels, but the change in water content from the time of field sampling to the level at full saturation tended to be higher in poplars and early-successional systems. All systems had similar WFPS levels.

Soils from the two biodiverse systems, poplars and early succession, substantially differed from the other three systems in terms of their pore size distributions (Figure 1). These soils had higher abundance of 60–270 µm pores than the other three systems. The differences were particularly pronounced for pore sizes between 60 and 180 µm. The differences among corn and switchgrass systems in pore size distributions were not statistically significant, and even numerically the differences were very minor across the entire range of the studied pore sizes.
3.2 | Isotopic composition of N\textsubscript{2}O and percentage of N\textsubscript{2}O derived from denitrification

The ranges in δ\textsuperscript{15}N, δ\textsuperscript{18}O, and SP for all samples analyzed are −33.2 to −0.4, 16.4 to 40.7, and −6.2 to 10.6 ‰ (Supporting information Table S1). These low SP values are consistent with a strong domination of N\textsubscript{2}O production from bacterial denitrification (Ostrom & Ostrom, 2011). Based on these SP values, the proportion of N\textsubscript{2}O derived from bacterial denitrification ranged from 55% to 91.9% or 64.3 to over 100% depending on whether the conservative or nonconservative model is used (Supporting information Table S1).

3.3 | Effect of bioenergy systems on N\textsubscript{2}O

The bioenergy system effect on the proportion of N\textsubscript{2}O generated via bacterial denitrification (N\textsubscript{2}O-BD) was not statistically significant (p = 0.11). Numerically, corn with cover crop and early succession had lower N\textsubscript{2}O-BD levels than...
the other three systems (Figure 2a). Likewise, the bioenergy systems did not differ in the cumulative amount of N₂O emitted during 3 day incubations (CumN₂O). Numerically, the two biodiverse systems had somewhat lower CumN₂O values than the low-diversity systems (Figure 2b), which is consistent with past field observations from this experimental site (Oates et al., 2016).

3.4 | Soil variables associated with proportion of N₂O generated via denitrification and with cumulative emitted N₂O

The five bioenergy systems separate into two distinct groups in terms of the relationships between soil characteristics and two N₂O variables, that is, N₂O-BD and CumN₂O. The first group consists of the two corn systems and switchgrass, which had lower soil C, low porosity, and low abundance of pores in 60–270 µm range. The second group consists of two biodiverse systems, poplars and early succession, which have high soil C levels, high porosity, and high abundances of pores in 60–270 µm range. Since the main management difference between these two groups is the diversity of their plant communities, we will refer to them as low-diversity and high-diversity systems, respectively.

In soils of low-diversity systems N₂O-BD was positively correlated with Vol-180 and negatively correlated with presence of pores in 30–120 µm size range (Table 2). The relationship between N₂O-BD and Vol-180 was non-linear with quadratic regression explaining 22% of N₂O-BD variability (Figure 3a).

In soils of high-diversity systems, N₂O-BD was related to WFPS and, especially, to saturation of small pores (Table 2), however not to Vol-180 (Figure 3b). N₂O-BD in the high-diversity systems’ soil was negatively related to the change in water content from field level to saturation (Table 2). Lower N₂O-BD levels were observed in the soil cores where the change in water content was large, while higher N₂O-BD levels tended to be present in the cores where such change was small. To some extent, this trend was present in low-diversity systems as well, excluding continuous corn, the system where a very narrow and low range of water content change values was observed (Figure 4).

In low-diversity systems, the relationships of CumN₂O with soil variables were very similar to those of N₂O-BD, and included positive correlation with Vol-180 and negative correlations with presence of pores in 30–60 µm size range (Table 2). In high-diversity systems, CumN₂O was strongly associated with several soil variables, including some of the studied water content variables, Vol-180, and pores in the 30–150 µm size group. While in both low- and high-diversity systems, CumN₂O was positively related
to Vol-180, WFPS, and saturation of small pores, the relationships were much stronger in high-diversity systems (Table 2, Figure 3).

Contrary to the expectations, neither the total POM nor connected or unconnected POM was related to N₂O-BD in either low- or high-diversity systems (Table 2). In low-diversity systems, CumN₂O was not associated with either total POM or connected/unconnected POM. However, in high-diversity systems, the unconnected POM was strongly positively correlated with CumN₂O (Figure 5).

The relationships between the two studied N₂O variables themselves, that is, N₂O-BD and CumN₂O, markedly differed between the low- and high-diversity systems (Figure 6). In low-diversity systems, the emitted CumN₂O linearly increased as N₂O-BD increased from 60% to 80%, and slightly decreased as N₂O-BD increased to 90%. In high-diversity systems, CumN₂O was not related to N₂O-BD at the range 60%–80%. Note that N₂O-BD values >80% were not observed in high-diversity systems.

### 3.5 Effect of moisture regime

Numerically, the mean values of the N₂O-BD were the highest in Dry, followed by WetWC and WetPr water treatments, but the differences were not statistically significant (Figure 7a). The highest amount of N₂O was emitted from the Dry treatment (drying of wet soil), followed by WetWC (wetting dry soil to the same water content as Dry), and the lowest in WetPr (wetting dry soil to pressure similar to that of Dry, but higher than WetWC; Figure 7b).

### 4 Discussion

After 9 years of implementation, the bioenergy systems diverged into two distinct groups not only in terms of their organic matter and pore characteristics, but also in the factors contributing to N₂O production and emission. The latter is reflected by the differences between these two groups in terms of strengths and directions of the relationships between soil characteristics and N₂O variables. Specifically, in the low-diversity systems, corn and switchgrass, which had low C and low pore abundances, the emission of N₂O was strongly associated with the proportion of N₂O generated via bacterial denitrification. There both the proportion of N₂O generated via bacterial denitrification and the N₂O emission were greater at greater volumes of poorly aerated soil (Vol-180). However, water and pore characteristics explained only a small portion of variability in N₂O emissions in these systems. On the contrary, in the high-diversity systems, poplar and early succession, which had high C and high pore abundances, N₂O emission was not related to the proportion of N₂O generated via bacterial denitrification. Yet, a number of water and pore characteristics, notably Vol-180 and the abundance of unconnected POM, explained a substantial amount of variability in N₂O emissions in high-diversity systems.

Our data supported the hypothesis that long-term implementation of biofuel crops affects soil pore and water characteristics (Figure 1 and Table 1). Based on their pore characteristics, low- and high-diversity systems can be regarded, respectively, as those prone to high and low oxygen deficiency. Due to higher presence of >30 µm pores and lower Vol-180, the high-diversity systems had more favorable conditions for soil aeration and gas diffusion. On average, 88%–96% of POM in high-diversity systems was connected to the atmosphere by visible pores. On the contrary, the lower presence of >30 µm pores and higher Vol-

---

**TABLE 2** Correlation coefficients of proportion of N₂O generated via bacterial denitrification, N₂O-BD, and cumulative emitted N₂O, CumN₂O, with the selected soil characteristics

| Water content (WC) variables | Low diversity | High diversity |
|------------------------------|---------------|---------------|
| WC before incubation         | 0.14          | -0.11         |
| WFPS                         | 0.36          | 0.21          |
| Total porosity               | 0.26          | -0.23         |
| Change in WC from field to saturation | 0.33 | 0.09 | 0.66 | 0.52 |

| CT image-based variables     | Low diversity | High diversity |
|------------------------------|---------------|---------------|
| Pores <30 µm                 | -0.14         | 0.14          |
| Pores >30 µm                 | 0.07          | -0.47         |
| Saturation pores <30 µm      | 0.33          | 0.66          |
| Saturation pores >30 µm      | 0.18          | 0.01          |
| Vol-180                      | 0.35          | 0.48          |
| POM total                    | -0.13         | 0.14          |
| POM connected by >30 µm pores| -0.02         | 0.11          |
| POM unconnected by >30 µm pores| -0.20   | 0.19          |

| Pores of average radius (µm) | Low diversity | High diversity |
|------------------------------|---------------|---------------|
| 30                           | -0.42         | -0.48         |
| 60                           | -0.39         | -0.53         |
| 90                           | -0.27         | -0.48         |
| 120                          | -0.17         | -0.35         |
| 150                          | -0.11         | -0.22         |
| 180                          | -0.02         | -0.14         |

Notes: Correlation analysis was conducted separately in corn systems and switchgrass (low diversity) (n = ~24) and poplar and early-successional (high diversity) (n = ~16) systems. Correlation coefficients in bold and italic are significantly different from zero at p < 0.05 and p < 0.1, respectively.
180 of the low-diversity systems suggest greater proneness to anoxic conditions. On average, only 70%–80% of POM in low-diversity systems was connected to the atmosphere by visible pores. Moreover, soil moisture conditions at the time of sampling demonstrated that indeed, low-diversity systems were less aerated, requiring smaller changes in water content at the time of sampling to reach full saturation (Table 1). This suggests that for a long time before the sampling, that is, during fall and winter months when evapotranspiration was low, the conditions within low-diversity systems could have been potentially more conducive to denitrification than in the high-diversity systems.

The results only partially supported the hypothesis regarding the importance of water retention hysteresis for N₂O emissions. It appeared that in this study, the actual water content, and not the mode in which it was reached, that is, wetting versus drying, mattered the most. The N₂O emissions from the soil that was dried and then brought to −10 kPa pressure were significantly lower than those from the soil that was dried to −10 kPa pressure after saturation as well as from the soil that, after drying, was wetted to the same water content.

We formulated a path model to relate aeration conditions, history of saturation, bacterial denitrification, and N₂O emissions and fitted it separately to data from low-diversity and high-diversity systems (Figure 8). The model hypothesizes that the aeration conditions (represented by Vol-180) and a history of saturation (represented by the change in water content from field to saturation) influence N₂O-BD directly and influence CumN₂O both directly and indirectly through their effect on N₂O-BD.

### 4.1 Soil characteristics related to proportion of N₂O produced via bacterial denitrification

The effect of anoxic conditions on N₂O-BD differed in low-diversity and high-diversity systems. In low-diversity systems related to proportion of N₂O produced via bacterial denitrification.
systems, lower aeration directly translated into lower N₂O-BD as indicated by (a) the negative relationship of N₂O-BD with abundance of 30–120 µm pores (Table 2), (b) the positive relationship of N₂O-BD with Vol-180 (Figure 3a, Table 2), and (c) the significant positive direct effect of Vol-180 on N₂O-BD (Figure 8). This result is consistent with dominance of bacterial denitrification in soil with higher water content and lower aeration reported by Well, Kurganova, Gerenyu, and Flessa (2006). Kravchenko et al. (2017) observed that in the absence of incorporated plant leaves, the proportion of N₂O from bacterial denitrification was higher in the soil with prevalence of small (<10 µm) pores, the outcome driven by less aerated conditions in soils dominated by small pores and consistent with the results from low-diversity systems of this study. However, none of the indicators of low aeration associated with N₂O-BD in low-diversity systems were related to N₂O-BD in high-diversity systems (Figure 8).

Contrary to the expectations, the presence of POM was not related to N₂O-BD in either low- or high-diversity systems. Kravchenko et al. (2017) reported that when plant leaves were added to the soil, the proportion of N₂O generated via denitrification was higher in the soil with prevalence of large (>35 µm) pores than in the soil with prevalence of small (<10 µm) pores. They explained this result by formation of local anoxic conditions not within the soil, but within the decomposing leaves themselves. Hence, we hypothesized that in the soils of high-diversity systems, due to their higher amounts of POM and large pores, N₂O-BD also would be positively associated with POM and pore abundance. Yet, this hypothesis was not supported by the data and greater anoxic conditions, which could be surmised to occur within POM fragments due to enhanced decomposition (Kravchenko et al., 2017; Negassa et al., 2015), did not translate into greater N₂O production via bacterial denitrification.

The historic conditions in terms of water content levels preceding soil sampling appeared to be one of the few soil
characteristics significantly associated with N₂O-BD in high-diversity systems. In high-diversity systems, the change in the water content at the time of sampling needed to reach full saturation was negatively correlated with N₂O-BD (Table 2), and in the studied path model, it had statistically significant negative effect on N₂O-BD (Figure 8). Note that in the low-diversity systems, these associations also had a negative sign, but were not statistically significant. The closer the soil was to full saturation at the time of sampling, the greater the proportion of N₂O generated via bacterial denitrification. This relationship suggests that historic presence of denitrification-favorable conditions plays a positive role in current denitrification processes. It can be inferred that if the soil was held for a long period of time at conditions close to saturation, then denitrifying communities may have proliferated and expanded from small pores and aggregate centers, where they typically reside (Ebrahimi & Or, 2015; Tiedje, Sexstone, Parkin, Revsbech, & Shelton, 1984). Soil samples for this study were collected in February, after ~3 months of high soil moisture levels. Their denitrifying communities were not likely affected by sampling or sample preprocessing; thus, they continued to actively function during the incubation experiment of this study. The potential importance of adaptation history in defining the ability of microbial communities to produce N₂O has been expressed before (Jungkunst, Freibauer, Neufeldt, & Bareth, 2006; Krause et al., 2017; Lagomarsino et al., 2016), while the history of soil moisture conditions was found to play an unexpectedly greater role than current soil water levels for other soil processes, for example, soil respiration (Smith, 2017).

### 4.2 Soil characteristics related to N₂O emission

Despite some similarities between low- and high-diversity systems in terms of associations between emitted N₂O and the soil aeration-related variables, overall, the two systems differed substantially, and the difference was most remarkable for CumN₂O versus N₂O-BD relationship. In the low-diversity systems, greater proportion of N₂O from bacterial denitrification directly translated into greater N₂O emissions (Figure 6a, Figure 8). The magnitude of anoxic conditions, as indicated by (a) greater area of unaerated soil volume Vol-180 and (b) lower presence of 30–60 µm pores, appeared to be the main physical factor driving both the proportion of bacterial denitrification and N₂O emissions in these soils. In the high-diversity systems, bacterial denitrification had a statistically nonsignificant, but numerically also positive, direct effect on emitted N₂O (Figure 8). However, the straightforward pathway of poor aeration leading to greater contribution from bacterial denitrification to greater N₂O emissions, which was clearly observed in low-diversity systems, was not present in high-diversity soils.

In soils of high-diversity systems, poor aeration conditions led to substantially greater N₂O emissions directly. The positive effect of poor aeration was manifested through (a) negative relationship of CumN₂O with presence of 30–120 µm pores (Table 2), (b) positive relationship of CumN₂O with Vol-180 and with the POM unconnected to the atmosphere by visible pores (Figure 3b and Figure 5), and (c) positive relationship of CumN₂O with WFPS and saturation of small pores (Table 2). Yet, while poor aeration did favor increased N₂O emissions, it did so without a concomitant increase in the proportion of N₂O produced via bacterial denitrification. In fact, the direct effect of Vol-180 on CumN₂O (0.99) and its indirect effect on CumN₂O via N₂O-BD mediation (−0.31) (calculated as a product of −0.85 and 0.46) had opposite signs. This inconsistency suggests that in these soils, other anaerobic processes besides bacterial denitrification contributed to N₂O production, perhaps fungal denitrification. The Sr of N₂O produced by fungi has been shown to be ~37‰ (Rohe et al., 2014; Sutka, Adams, Ostrom, & Ostrom, 2008), which is substantially greater than the values of −10 to 0‰ associated with production from bacterial denitrification (Frame & Casciotti, 2010; Sutka et al., 2006; Toyoda & Yoshida, 1999). Fungi have been demonstrated to denitrify at more oxic conditions than bacteria and to be a substantial contributor to N₂O production, especially in woody soils, but also in grasslands and soils of agricultural systems (Chen

![FIGURE 8](image-url) Path analysis model representing effects of the volume of poorly aerated soil, Vol-180, and historic absence of anoxic conditions (WCchange) on proportion of N₂O generated by bacterial denitrification (N₂O-BD) and on the total amount of emitted N₂O (CumN₂O). Coefficients in red are for low-diversity systems, marked by ** (p < 0.05)
straightforward associations between N₂O emissions and then fungal denitrification can be expected to result in more N₂O is the terminal product of denitrification by fungi, (Miller, Baggs, & Johnson, 2011; Sutka et al., 2008). Since bioenergy systems with low and high plant diversities. lack the capability of N₂O to N₂ reduction (Prendergast-Miller, Baggs, & Johnson, 2011; Sutka et al., 2008). Since N₂O is the terminal product of denitrification by fungi, then fungal denitrification can be expected to result in more straightforward associations between N₂O emissions and the factors contributing to it, as the additional nonlinear effect of reduced N₂O production via its reduction to N₂ is eliminated. This could be a potential explanation for better predictive powers of soil water and pore characteristics observed in high-diversity systems of this study. It should be also noted that N₂O reduction to N₂ was more likely to take place in poorly aerated soils of low-diversity systems, and, due to tendency for fungi to live in larger pores with greater air diffusion, was likely more affecting N₂O of bacterial than fungal origin. This would explain lack of differences in the average values of CumN₂O and N₂O-BD among the studied systems. Further assessment of fungal denitrification and fungal presence in these soils is needed to test this hypothesis.

It should be also noted that reduction of N₂O can alter SP; however, reduction shifts the relationship between δ¹⁸O and δ¹⁵N and δ¹⁸O and δ¹⁵N⁴ shift toward 2.6 and 1.9, respectively, when reduction occurs (Ostrom et al., 2006; Jinuntuya-Nortman et al, 2008). Based on the data in Supporting information Table S1, we observed relationships between δ¹⁸O and δ¹⁵N and δ¹⁸O and δ¹⁵N⁴ of 0.45 and 0.46, respectively, that are not consistent with a strong influence of N₂O reduction in our samples (Ostrom & Ostrom, 2011). Further, as discussed in the Introduction section, reduction of N₂O equal to 10% of its production will only shift Sp by 0.7 %e (Oddyke et al., 2009), which has a minor effect on our estimates of the importance of bacterial denitrification.

The findings indicated that several years of implementing bioenergy systems with contrasting plant diversities led to substantial changes in soil pore characteristics, along with soil C and N levels. These changes were likely one of the contributors to the observed differences in associations between N₂O emissions and soil microscale variables in bioenergy systems with low and high plant diversities. Contrary to the expectations, none of the studied pore or POM characteristics worked as an effective predictor of N₂O emissions across the entire set of the systems. However, saturation of <30 µm pores, presence of air-filled 30–90 µm pores, size of poorly aerated soil volume, and volume of POM unconnected to the atmosphere appeared to be feasible microscale soil predictors of N₂O emissions in soils from bioenergy systems with high plant diversity. Yet, in the systems with low plant diversity, N₂O production via bacterial denitrification appeared to be the only feasible predictor of N₂O emissions, while predictive capabilities of microscale pore and POM data were relatively weak. The possible cause is that long-term differences in implementing contrasting bioenergy systems may have affected the relative contributions of different processes, for example, bacterial and fungal denitrification and nitrification, to N₂O production and further reduction. Differences in influences from physical microscale soil characteristics on these processes likely led to the observed differences in capabilities of different soil characteristics in predicting N₂O emissions.

ACKNOWLEDGEMENTS

This work was funded in part by National Science Foundation’s Long-Term Ecological Research Program (DEB 1027253) and by the National Science Foundation’s Geobiology Program 460 (Award #1630399). This material is partly based upon work supported by the US Department of Energy, Office of Science, Office of Biological and Environmental Research, under Award Number DESC0018409, and work funded by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494). The work of A. Kravchenko was supported by DAAD-German Academic Exchange Service's program "Research Stays for University Academics and Scientists, 2017" (57314018) and by the Research Award from the Alexander von Humboldt Foundation.

ORCID

Alexandra N. Kravchenko [http://orcid.org/0000-0001-5920-927X]

REFERENCES

Balaine, N., Clough, T. J., Beare, M. H., Thomas, S. M., Meenken, E. D., & Ross, J. G. (2013). Changes in relative gas diffusivity explain soil nitrous oxide flux dynamics. Soil Science Society of America Journal, 77, 1496–1505. https://doi.org/10.2136/sssa.j2013.04.0141

Ball, B. C. (2013). Soil structure and greenhouse gas emissions: A synthesis of 20 years of experimentation. European Journal of Soil Science, 64, 357–373. https://doi.org/10.1111/ejss.12013
Kravchenko, A. N., Negassa, W. C., Guber, A. K., & Rivers, M. L. (2015). Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. Soil Biology & Biochemistry, 84, 107–115. https://doi.org/10.1016/j.soilbio.2015.02.022
Heil, J., Liu, S. R., Vereecken, H., & Bruggemann, N. (2015). Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. Soil Biology & Biochemistry, 84, 107–115. https://doi.org/10.1016/j.soilbio.2015.02.022
Heil, J., Wolf, B., Bruggemann, N., Emmenegger, L., Tuzson, B., Vereecken, H., & Mohn, J. (2014). Site-specific N-15 isotopic signatures of abiotically produced N2O. Geochimica Et Cosmochimica Acta, 139, 72–82.
Henault, C., Grossel, A., Mary, B., Roussel, M., & Leonard, J. (2012). Nitrous oxide emission by agricultural soils: A review of spatial and temporal variability for mitigation. Pedosphere, 22, 426–433. https://doi.org/10.1016/S1002-0160(12)60029-0
Huang, X. D., Grace, P., Mengersen, K., & Weier, K. (2011). Spatio-temporal variation in soil derived nitrous oxide emissions under sugarcane. Science of the Total Environment, 409, 4572–4578. https://doi.org/10.1016/j.scitotenv.2011.07.044
Jesus, E. D., Liang, C., Quensen, J. F., Susilawati, E., Jackson, R. D., Balser, T. C., & Tiedje, J. M. (2016). Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. Global Change Biology, 22, 481–494. https://doi.org/10.1111/gcb.12289
Jinjuntyua-Nortman, M., Sutka, R. L., Ostrom, P. H., Gandhi, H., & Ostrom, N. E. (2008). Isotopologue fractionation during microbial reduction of N2O within soil mesocosms as a function of water-filled pore space. Soil Biology & Biochemistry, 40, 2273–2280. https://doi.org/10.1016/j.soilbio.2008.05.016
Johnson, J. M. F., & Barbour, N. W. (2016). Nitrous oxide emission and soil carbon sequestration from herbaceous perennial biofuel feedstocks. Soil Science Society of America Journal, 80, 1057–1070. https://doi.org/10.1002/saj.2015.12.0436
Jungkunst, H. F., Freibauer, A., Neufeldt, H., & Bareth, G. (2006). Nitrous oxide emissions from agricultural land use in Germany—A synthesis of available annual field data. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrhund Und Bodenkunde, 169, 341–351. https://doi.org/10.1002/jpln.200521954
Keiluweit, M., Gee, K., Denney, A., & Fendorf, S. (2018). Anoxic microsites in upland soils dominantly controlled by clay content. Soil Biology & Biochemistry, 118, 42–50. https://doi.org/10.1016/j.soilbio.2017.12.002
Keiluweit, M., Nico, P. S., Kleber, M., & Fendorf, S. (2016). Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils? Biogeochemistry, 127, 157–171. https://doi.org/10.1007/s10533-015-0180-6
Keiluweit, M., Wanzek, T., Kleber, M., Nico, P., & Fendorf, S. (2017). Anaerobic microsites have an unaccounted role in soil carbon stabilization. Nature Communications, 8. https://doi.org/10.1038/s41467-017-01406-6
Krause, H. M., Thomar, C., Eschenbach, W., Well, R., Mäder, P., Behrens, S., ... Gattinger, A. (2017). Long term farming systems affect soils potential for N2O production and reduction processes under denitrifying conditions. Soil Biology & Biochemistry, 114, 31–41. https://doi.org/10.1016/j.soilbio.2017.06.025
Kravchenko, A. N., Negassa, W. C., Guber, A. K., & Rivers, M. L. (2015). Protection of soil carbon within macro-aggregates depends on intra-aggregate pore characteristics. Scientific Reports, 5, 16261. https://doi.org/10.1038/srep16261

Barnard, R., Leadley, P. W., & Hungate, B. A. (2005) Global change, nitrification, and denitrification: A review. Global Biogeochemical Cycles, 19. https://doi.org/10.1029/2004GB002282.
Buchwald, C., Grabb, K., Hansel, C. M., & Wankel, S. D. (2016). Constraining the role of iron in environmental nitrogen transformations: Dual stable isotope systematics of abiotic NO2-reduction by Fe(II) and its production of N2O. Geochimica Et Cosmochimica Acta, 186, 1–12. https://doi.org/10.1016/j.gca.2016.04.041
Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: How well do we understand the processes and their controls? Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 368, 20130122.
Chen, H. H., Mothapo, N. V., & Shi, W. (2014). The significant contributions of fung to N2O production across diverse ecosystems. Applied Soil Ecology, 73, 70–77. https://doi.org/10.1016/j.apsoil.2013.08.011
Chen, H. H., & Shi, W. (2017). Opening up the N2O-producing fungal community in an agricultural soil with a cytochrome p450nor-based primer tool. Applied Soil Ecology, 119, 392–395. https://doi.org/10.1016/j.apsoil.2017.07.022
Crenshaw, C. L., Lauber, C., Sinsabaugh, R. L., & Stavely, L. K. (2008). Fungal control of nitrous oxide production in semiarid grassland. Biogeochemistry, 87, 17–27. https://doi.org/10.1007/s10533-007-9165-4
Ebrahimi, A., & Or, D. (2015). Hydration and diffusion processes shape microbial community organization and function in model soil aggregates. Water Resources Research, 51, 9804–9827. https://doi.org/10.1002/2015WR017565
Fowler, D., Pilegaard, K., Sutton, M. A., Ambus, P., Raivonen, M., Duyzer, J., & Granier, C. (2009). Atmospheric composition change: Ecosystems-atmosphere interactions. Atmospheric Environment, 43, 5193–5267. https://doi.org/10.1016/j.atmosenv.2009.07.068
Frame, C. H., & Casciotti, K. L. (2010). Biogeochecmical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. Biogeoosciences, 7, 2695–2709. https://doi.org/10.5194/bg-7-2695-2010
Grabb, K. C., Buchwald, C., Hansel, C. M., & Wankel, S. D. (2017). A dual nitrite isotopic investigation of chemodenitrification by mineral-associated Fe(II) and its production of nitrous oxide. Geochimica Et Cosmochimica Acta, 196, 388–402. https://doi.org/10.1016/j.gca.2016.10.026
Groffman, P. M., Butterbach-Bahl, K., Fulweiler, R. W., Gold, A. J., Morse, J. L., Stander, E. K., ... Vidon, P. (2009). Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. Biogeochemistry, 93, 49–77. https://doi.org/10.1007/s10533-008-9277-5
Guo, X. B., Drury, C. F., Yang, X. M., Reynolds, W. D., & Fan, R. Q. (2014). The extent of soil drying and rewetting affects nitrous oxide emissions, denitrification, and nitrogen mineralization. Soil Science Society of America Journal, 78, 194–204. https://doi.org/10.2136/sssaj2013.06.0219
Harrison-Kirk, T., Beare, M. H., Meenken, E. D., & Condron, L. M. (2013). Soil organic matter and texture affect responses to dry/wet cycles: Effects on carbon dioxide and nitrous oxide emissions. Soil Biology & Biochemistry, 57, 43–55. https://doi.org/10.1016/j.soilbio.2012.10.008
Haslun, J. A., Ostrom, N. E., Hegg, E. L., & Ostrom, P. H. (2018). Estimation of isotope variation of N2O during denitrification by Pseudomonas aureofaciens and Pseudomonas chlororaphis: Implications for N2O source apportionment. Biogeoosciences, 15, 3873–3882.
emissions during soil drying. Vadose Zone Journal, 14. https://doi.org/10.2136/vzj2014.12.0177

Robertson, G. P., & Hamilton, S. K. (2015) Long-term ecological research in agricultural landscapes at the Kellogg Biological Station LTER site: conceptual and experimental framework. In: S. K. Hamilton, J. E. Doll, & G. P. Robertson (eds) The ecology of agricultural landscapes: long-term research on the path to sustainability. New York, NY: Oxford University Press.

Robertson, G. P., Paul, E. A., & Harwood, R. R. (2000). Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Science, 289, 1922–1925. https://doi.org/10.1126/science.289.5486.1922

Robertson, G. P., & Tiedje, J. M. (1987). Nitrous oxide sources in aerobic soils - nitrification, denitrification and other biological processes. Soil Biology & Biochemistry, 19, 187–193. https://doi.org/10.1016/0038-0717(87)90080-0

Rohe, L., Anderson, T. H., Braker, G., Flessa, H., Giesemann, A., Lewicka-Szczechak, D., … Well, R. (2014). Dual isolate and isotope signatures of nitrous oxide from fungal denitrification - a pure culture study. Rapid Communications in Mass Spectrometry, 28, 1893–1903. https://doi.org/10.1010/rcm.6975

Sanford, G. R., Oates, L. G., Jasrotia, P., Thelen, K. D., Robertson, G. P., & Jackson, R. D. (2016). Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. Agriculture Ecosystems & Environment, 216, 344–355.

Schindelin, J., Arganda‐Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Tinevez, J. Y. (2012). Fiji: An open-source platform for biological-image analysis. Nature Methods, 9, 676–682. https://doi.org/10.1038/nmeth.2019

Schurgers, G., Dorsch, P., Bakken, L., Leffelaar, P., & Haugen, L. E. (2006). Modelling soil anaerobiosis from water retention characteristics and soil respiration. Soil Biology & Biochemistry, 38, 2637–2644. https://doi.org/10.1016/j.soilbio.2006.04.016

Sexstone, A. J., Revsbech, N. P., Parkin, T. B., & Tiedje, J. M. (1985). Direct measurement of oxygen profiles and denitrification rates in soil aggregates. Soil Science Society of America Journal, 49, 645–651.

Smith, K. A. (2017). Changing views of nitrous oxide emissions from agricultural soil: Key controlling processes and assessment at different spatial scales. European Journal of Soil Science, 68, 137–155. https://doi.org/10.1111/ejss.12409

Sprunger, C. D., Oates, L. G., Jackson, R. D., & Robertson, G. P. (2017). Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA. Biomass & Bioenergy, 105, 248–258.

Sprunger, C. D., & Robertson, G. P. (2018). Early accumulation of active fraction soil carbon in newly established cellulosic biofuel systems. Geoderma, 318, 42–51. https://doi.org/10.1016/j.geoderma.2017.11.040

Sutka, R. L., Adams, G. C., Ostrom, N. E., & Ostrom, P. H. (2008). Isotopologue fractionation during N₂O production by fungal denitrification. Rapid Communications in Mass Spectrometry, 22, 3989–3996.

Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Breznak, J. A., Gandhi, H., Pitt, A. J., & Li, F. (2006). Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. Applied and Environmental Microbiology, 72, 638–644. https://doi.org/10.1128/AEM.72.1.638-644.2006

Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Gandhi, H., & Breznak, J. A. (2004). Nitrogen isotopomer site preference of N₂O produced by Nitrosomonas europaea and Methylococcus capsulatus Bath (vol 18, pg 1411, 2004). Rapid Communications in Mass Spectrometry, 18, 1411–1412. https://doi.org/10.1010/rcm.1482

Tiedje, J. M., Sexstone, A. J., Parkin, T. B., Revsbech, N. P., & Sherlock, D. R. (1984). Anaerobic processes in soil. Plant and Soil, 76, 197–212. https://doi.org/10.1007/BF02205580

Toyoda, S., & Yoshida, N. (1999). Determination of nitrogen isotopomers of nitrous oxide on a modified isotope ratio mass spectrometer. Analytical Chemistry, 71, 4711–4718. https://doi.org/10.1021/ac9904563

Van Groenigen, K. J., Osenberg, C. W., & Hungate, B. A. (2011). Increased soil emissions of potent greenhouse gases under increased atmospheric CO₂. Nature, 475, 214–U121. https://doi.org/10.1038/nature10176

Vogel, H. J., Weller, U., & Schluter, S. (2010). Quantification of soil structure based on Minkowski functions. Computers & Geosciences, 36, 1236–1245. https://doi.org/10.1016/j.cageo.2010.03.007

Walter, K., Don, A., & Flessa, H. (2015). Net N₂O and CH₄ soil fluxes of annual and perennial bioenergy crops in two central German regions. Biomass & Bioenergy, 81, 556–567. https://doi.org/10.1016/j.biombioe.2015.08.011

Wang, W., Kravchenko, A. N., Smucker, A. J. M., Liang, W., & Rivers, M. L. (2012). Intra-aggregate pore characteristics: X-ray computed microtomography analysis. Soil Science Society of America Journal, 76, 1159–1171. https://doi.org/10.2136/ssaj2011.0281

Well, R., Kurganova, I., De Genyenu, V. L., & Flessa, H. (2006). Isotopomer signatures of soil-emitted N₂O under different moisture conditions - A microcosm study with arable loess soil. Soil Biology & Biochemistry, 38, 2923–2933. https://doi.org/10.1016/j.soilbio.2006.05.003

Wightman, J. L., Duxbury, J. M., & Woodbury, P. B. (2015). Land quality and management practices strongly affect greenhouse gas emissions of bioenergy feedstocks. Bioenergy Research, 8, 1681–1690. https://doi.org/10.1007/s12155-015-9620-3

Wuensch, K. L. (2016). An introduction to path analysis. Retrieved from https://core.ecu.edu/psyc/wuenschk/MV/SEM/Path.pdf on 07/10/2018

Zhu-Barker, X., Cavazos, A. R., Ostrom, N. E., Horwath, W. R., & Glass, J. B. (2015). The importance of abiotic reactions for nitrous oxide production. Biogeochemistry, 126, 251–267. https://doi.org/10.1007/s10533-015-0166-4

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.