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Plant diversity decreases potential nitrous oxide emissions from restored agricultural soil

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ABSTRACT

Plant diversity has been shown to mitigate nitrous oxide (N_2O) emissions from soil in some ecosystems. We tested the generality of this diversity effect on ecosystem functioning, as an application of ecological theory by quantifying potential nitrous oxide emissions in grasslands restored from agriculture containing high and low diversity. We performed an initial plant survey to develop the study design from quadrat analyses (used to determine sampling frame size that maximizes variance in plant community structure), semivariogram analyses of plant community PCA scores (used to determine minimum distance between sampling frames), and upper and lower quartiles of plant diversity (Shannon's diversity). We sampled high (per field n = 6, total n = 60) and low (per field n = 6, total n = 60) diversity (high diversity: mean Shannon diversity index = 1.69; low diversity: mean Shannon diversity index = 0.73) plant communities within 10 to 12 y old restorations co-located at Nachusa Grasslands (Franklin Grove, IL, USA). We found potential N_2O emissions were nearly seven times greater in low diversity compared to high diversity communities. Inorganic N availability, relative amounts of nitrification and denitrification, and water extractable C did not explain difference in potential N_2O emission from soil with contrasting plant diversity. Although the mechanism for large variation in this ecosystem function corresponding with biodiversity remains undetermined, this work shows that management for plant diversity may reduce N_2O emissions from soil.

1. Introduction

Agricultural soil is a major anthropogenic source of nitrous oxide (N_2O; Skiba et al.,1993, Zhang et al., 2014), a greenhouse gas that is 265x as potent as CO_2 (IPCC 2018). More N_2O is produced on a per mole basis during denitrification than nitrification, but anaerobic conditions are required. In agricultural soils, which are often drained and less likely to be anaerobic, nitrification can be the major source of N_2O (Skiba et al., 1993). A meta-analysis concluded that agricultural soils in reduced or no-till for >10 years produced less area-scaled N_2O emissions as compared to conventional tillage (van Kessel et al., 2013), suggesting degraded soils produce more N_2O emissions. Prairie restoration from agricultural (row-crop) conditions has been shown to improve soil structure, increase microbial biomass and richness, and reduce nutrient availability (Baer et al., 2003, Bach et al., 2010, Baer et al., 2010). These changes belowground, coupled with increasing plant diversity relative to agricultural systems, have been shown to reduce N_2O emissions from soil (Scott et al., 2019). Nitrous oxide emissions from soil are also lower under diverse (relative to less diverse) plant communities in multiple ecosystems including constructed wetlands (Han et al., 2017), experimental grasslands (Niklaus et al., 2016), undisturbed grasslands (Piñeiro-Guerra et al., 2019), and grasslands managed for grazing (Ribas et al., 2015), but no studies have examined whether high plant diversity communities in reconstructed grasslands reduce N_2O emissions compared to low diversity communities. Further, there has been little exploration of mechanisms responsible for lower N_2O emissions from soil in more diverse plant communities.

Nitrogen cycling in soil predominately occurs through microbial transformations and plant uptake. Plants compete with soil microbes for nitrogen mineralized from soil organic matter (SOM; Kaye and Hart 1997, Kuzyakov and Xu 2013, Liu et al., 2016). This SOM is depolymerized and N monomers are mineralized by a wide array of microbes to produce ammonium (NH_4^+; Schimel and Bennett 2004). Some NH_4^+ may volatize as ammonia gas (NH_3) under basic conditions and some will be

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oxidized (nitrified) to nitrite (NO_2^-) and then to nitrate (NO_3^-). Nitrification can be an autotrophic or a heterotrophic process, depending on the soil microbial community (Evans, 2007; van Groenigen et al., 2015). During nitrification, a small amount of NH_4^+ will be converted to N_2O. Nitrate is the more mobile form of N in many temperate ecosystems, but both NO_3^- and NH_4^+ can be taken up by plants. In the absence of O_2, NO_3^- can be used as an alternate electron acceptor and reduced to atmospheric nitrogen (N_2), a process known as denitrification. Greater plant uptake of NH_4^+ than NO_3^- can promote denitrification. Complete denitrification is a stepwise reduction from NO_3^-, NO_2^-, N_2O, and finally N_2. However, N_2O can be released during denitrification as some denitrifiers lack nitrous oxide reductase (Evans, 2007; van Groenigen et al., 2015). Additionally, abiotic reduction of NO_2^- can occur to produce nitrous oxide. When nitrifiers produce NO_2^- that is then reduced it is termed nitrifier denitrification (van Groenigen et al., 2015). Nitrification and denitrification can occur simultaneously in soil due to heterogeneity in aerobic and anaerobic microsites within soil aggregates (Stolk et al., 2011).

The relative amount of denitrification compared to nitrification can influence the amount of N_2O produced and can be measured with stable isotopes. Isotopic composition of N in soil is difficult to measure in the field, but can be determined with well-designed lab experiments (Evans, 2007). Several biological processes discriminate against ^{15}N isotopes and N fractionation observed in soil is typically expressed as $\delta^{15}N_{substrate} - \delta^{15}N_{product}$ (Evans, 2007). Nitrous oxide produced during nitrification is a product of two reactions: the oxidation of NH_2OH with NOH as a precursor and reduction of NO_2^- by nitrite reductase. Nitrous oxide produced during denitrification is an intermediate compound in the conversion of NO_3^- to N_2. Stable isotope signature of N_2O can change with site and season, and a greater fractionation factor occurs for nitrification as compared to denitrification (Barford et al., 1999; Yoshida, 1988). Thus, N_2O with relatively low $\delta^{15}N$ values suggest more nitrification, whereas more denitrification is indicated by relatively high $\delta^{15}N$ (Perez et al., 2000, 2001).

According to biodiversity-ecosystem function theory (BEF), plant diversity is predicted to promote ecosystem functioning through greater niche complementary (Tilman et al., 2014) or increased plasticity in resource use (Ashton et al., 2010). For example, diverse plant communities utilize more soil NO_3^- than less diverse plant communities (Tilman et al., 2001, Ashton et al., 2010, Johnson et al., 2016, Klopf et al., 2017). Plant diversity could influence N_2O emissions by altering availability of soil inorganic N or soil organic C. In addition to diversity effects, plant identity effect can influence N_2O emissions from soil as several plant functional traits including specific leaf area, leaf dry matter content, and specific root length are negatively associated with emissions (Abalos et al., 2018). Nitrous oxide production from soil can be limited by organic carbon (an electron donor source; Tiedje et al., 1984) and available nitrogen (Sotomayor and Rice 1995, Scott et al., 2019), but the influence of plant diversity on emissions from soils requires further study. Our objective was to determine if plant diversity influenced nitrous oxide emissions, and whether N_2O emissions were related to soluble organic C or differences in relative amount of nitrification and denitrification. We hypothesized that potential N_2O emissions from soil would be lower in plant communities with high diversity, corresponding with lower available N, higher soluble organic C, and lower nitrification rates.

2. Methods

2.1. Site Description

We quantified potential N_2O emissions at Nachusa Grasslands (Franklin Grove, IL, USA), a ~1600-hectare preserve of remnant grassland and woodland connected by restoration and managed by The Nature Conservancy, in early August 2017. Field sites were chosen from those identified and described in detail by Klopf et al. (2017). All sites were located in Lee and Ogle counties in northern Illinois. Mean annual precipitation in this area (1985–2009), was 968 mm and mean monthly temperature was 9.3 °C. Soils were similar and formed over glacial till with a loam texture (taxonomy in Table 1). All sites were managed regularly by prescribed burning (Table 1). *Bison bison* had access to one site (Site 3).

All sites were formerly cultivated and at the onset of restoration were sown with over 100 native species (Klopf et al., 2017). The fields were similar in age (10 to 12 years old; restored between 2005 and 2007) to reduce potential differences in N pools that change dynamically during restoration (Baer et al., 2002; Rosenzweig et al., 2016).

2.2. Sampling Design

An initial survey was conducted in July 2017 using 50 contiguous 0.25 m x 0.25 m (1/16 m^2) sampling frames near the center of each field along a temporary transect spanning 12.5 m along the length of the field. Percent cover was visually estimated for each vascular plant species rooted within the sampling frame. Initial survey results were analyzed with local quadrat variance, semivariogram, and quartile calculations and used to determine the appropriate sampling frame size and spatial arrangement (distance for statistical independence).

Appropriate sampling frame size was determined from the initial vegetation survey with three-term local quadrat variance (3TLQV; Hill 1973) analysis, which is less sensitive to global trends than two-term analogs, using Pattern Analysis, Spatial Statistics, and Geographic Exegesis version 2 (PASSaGE; Rosenberg and Anderson 2011) of the first axis scores from a principal component analysis (PCA). In this analysis the first distinct peak indicated the scale of maximum variance. The average distance indicated by 3TLQV analysis was used as the length and width of the sampling frame for all sites in the final sampling. This analysis suggested the scale of maximum variance in PCA first axis scores was 1.3 m. A 1-m^2 frame was used because this size frame is commonly used and is similar to the 3TQV result. A similar semivariogram analysis was conducted with the R package gstat (Pebesma 2004) to determine the distance between sampling frames of the same diversity level (range), so that replications were spatially independent. The range was 18.1 m. Upper and lower quartiles were determined from Shannon diversity (H) calculations of each sampling frame within a field. These H values were used to determine high- and low-diversity plant levels in sampling. The H cutoff values were < 0.89 for low diversity and > 1.33 for high diversity. This resulted in a high diversity level with a mean H value of 1.69 with a stand error of 0.045 and the low diversity level with a mean H value of 0.73 with a standard error of 0.031. Shannon diversity (hereafter referred to as plant diversity) was chosen as a diversity metric because it incorporates both richness and evenness.

Low-diversity ($n = 6$) and high-diversity ($n = 6$) plots were delineated within each of 5 restorations co-located at Nachusa Grasslands. In each restoration, we laid a 100 m transect and created 6 alternating secondary transects at a fixed distance apart (16.6 m) along the main transect (Fig. 1). A 1 m x 1 m sampling frame was moved laterally (starting at 19 m from the main transect) until appropriate high- and low-diversity plots were encountered. This sampling method allowed for greater than 18.1 m between sampling frames of the same diversity level (spatial independence based on initial survey). Percent cover within each plot was visually estimated and Shannon's diversity was determined. Differences in secondary transects were accounted for by blocking when the random effect was significant (Fig. 1).

2.3. Sampling and Response Variables

We removed ten 2 cm dia. cores to a depth of 10 cm from each sampling frame in early August. This sampling time was chosen because plants would be growing and presumably using soil nutrients. Soil was sampled to this depth because the surface 10 cm of soil are commonly evaluated in grassland restoration studies. The soil cores were then

Table 1
Site information. Soil types for sites were identified by Klopf et al., 2017. Soil taxonomy according to United states Department of Agriculture. Clay and sand content are ranges from a typical pedon according to National Cooperative Soil Survey official series descriptions Values for pH, NH_4–N, and NO_3–N refer to top 10 cm of soil profile.

Site	GPS Coordinate	Soil Type	Soil Taxonomy	Clay (%)	Sand (%)	Bison Grazing	Burn Frequency	Burned Last Year	Years Restored	pH	NH_4–N (g m^{-2})	NO_3–N (g m^{-2})
1	41.867014, -89.357091	Jasper Loam	Mesic typic Arguidoll	20–32	15–55	No	Annual	Yes	10	5.78	0.11	0.06
2	41.866767, -89.358301	Jasper Loam & Martinsville silt loam	Mesic typic Arguidoll & Mesic typic Hapludalf	20–33	15–55	No	Annual	Yes	11	5.96	0.13	0.08
3	41.896570, -89.352700	Jasper Loam	Mesic typic Arguidoll	20–32	15–55	Yes	Biennial	Yes	12	5.96	0.17	0.03
4	41.906721, -89.335929	Waukee Loam	Mesic typic Hapludoll	18–25	35–45	No	Biennial	No	10	5.69	0.10	0.05
5	41.904505, -89.329443	Waukee Loam	Mesic typic Hapludoll	18–25	35–45	No	Biennial	No	11	5.33	0.29	0.02

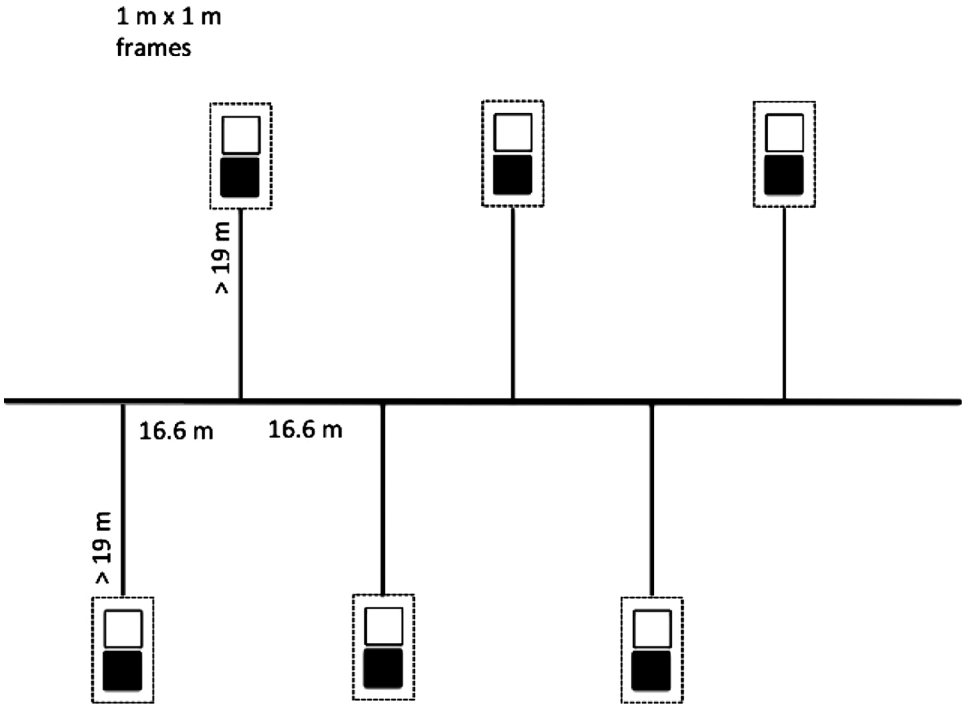

Fig. 1. Design at one restoration site (block). A main transect with alternating secondary transects every 16.6 m was delineated. One high diversity (black square) and one low diversity (white square) frame were established at least 19 m from the main transect at each secondary transect (replicate block; designated by dotted box).

composited for each frame and sieved (4 mm). Composited soil cores were refrigerated for less than one week until the short-term incubation experiment could be established. An intact 5.5 cm dia. core was also taken from half of the frames within a diversity level to determine bulk density so that concentrations could be expressed on a per area basis.

Water holding capacity of composited soil samples was determined by saturating a subsample (~20 g) of fresh soil, then allowing soil to drain by gravity for 16 hours in a sealed cooler with 100% humidity (Robertson et al., 1999). Water holding capacity was then calculated as the gravimetric water content of drained soils, determined by oven drying at 105° C. Bulk density of the soil was determined from drying the intact cores at 105° C. Soil pH (2:1 deionized water:air dried soil) was measured using a benchtop pH meter. Inorganic N was determined using a 2 N KCl extraction with colorimetric detection using OI Analytical Flow Solution IV [OI Analytical Corp., College Station, TX, USA]).

Lab incubations of soil composited at the frame level and adjusted to a standardized moisture level were used because spatio-temporal variability of N_2O emissions makes statistical analysis of *in situ* gas samples difficult with small sample sizes (Kravchenko and Robertson 2014). Soils adjusted to moisture levels consistent with maximum N_2O emissions were used, rather than denitrification potentials, because nitrification and denitrification can both contribute to N_2O emissions (Scott et al., 2019). While these lab potentials likely differ from *in situ* measurements, they are useful for determining the relative effect of plant diversity. To determine potential 1-day N_2O emissions under mixed aerobic and anaerobic conditions (Cheng et al., 2015), a subsample (~40 g dry equivalent weight) of the homogenized soil was placed in 250 ml flasks, adjusted to 40% water holding capacity (resulting in a narrow water-filled pore space range of 48.9%–60.6%) then incubated at 23°C in the dark (flask housed within a mason jar fit with septa) for 1 d. A 12 ml headspace gas sample was injected into 12 ml gas vials that had been flushed with He gas and vented to approximately 1 atm of pressure.

Natural abundance isotopic composition of N_2O emissions was then

determined with gas chromatography-isotope ratio mass spectrometry (GC-IRMS) at the University of California Davis Stable Isotope Facility. Low $\delta^{15}N\text{-}N_2O$ values indicate more nitrification relative to denitrification (Perez et al., 2000, 2001). This approach was used to estimate long-term relative amounts of nitrification and denitrification, rather than taking multiple gas samples over time. Gas samples were purged from Exetainer 12 ml glass soda vials (Labco Limited, Lapeter, UK) through a double-needle sampler into a helium carrier stream (20 mL/min) and analyzed with a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). The detection limit of N_2O with this setup is 150 picomoles with a 0.1 ‰ ^{15}N long-term standard deviation.

Because differences in N_2O could result from differences in labile organic C availability, we measured naturally occurring water extractable organic C values in the soil samples used to measure N_2O efflux (WEOC; Bai et al., 2014). To measure WEOC, a subsample (~10 g) of air-dried soil was placed into glass centrifuge tubes with 40 ml of deionized water. Tubes were shaken for 1 hour, and then centrifuged at 3600 rpm for 20 minutes. Effluent was then filtered using 0.4 µm HTTP Isopore Membrane Filters (Merk Millapore Ltd.). Filtrate was refrigerated until it could be analyzed with a total organic carbon analyzer (Shimadzu TOC-L, Shimadzu Corp., Kyoto, Japan).

2.4. Calculations and Statistical Analyses

To ensure high and low plant diversity communities were sampled, we tested if there was a difference in Shannon diversity values between high diversity and low diversity frames using linear mixed models with marginal *P* values and estimated marginal means. Linear mixed models were fit with the nlme package (Pinheiro et al., 2019) and estimated marginal means and associated standard errors were calculated with the emmeans package (Lenth 2019) in R (R core team 2019). An alpha level of 0.05 was used for all analyses. To meet the assumptions of homoscedasticity and normality of residuals, potential nitrous oxide was natural log transformed after adding 1 and $\delta^{15}N\text{-}N_2O$ was square root transformed after adding 8.19, so that all values were positive numbers prior to applying transformation. Blocking (random intercept) by replicate within site and only a site random effects were evaluated by Wald chi square test for each response. When replicate random effect was not significant, it was dropped from the model. The model of species richness response to diversity levels had blocking by replicate within site; all other models had blocking by site only. We also tested for differences between richness and Pielou's evenness, the two components of Shannon's diversity. We tested for an identity effect on potential N_2O emissions using percent cover of the nine most frequently occurring plant species as a predictor of natural log-transformed potential N_2O emissions in individual linear mixed models with site as a random intercept. Soil pH and extractable ammonium and nitrate were also compared using linear mixed models with site as a random intercept. Because soil pH and extractable N were likely to be correlated with isotopic composition of N_2O–N these variables these variables were not included in the path model.

Because N_2O can be affected by plant diversity and plant community composition in multiple ways, we developed a path model to evaluate impact of possible mechanisms. We used correspondence analysis (CA) scores, based on percent cover values for each species, from the vegan package (Oksanen et al., 2019) to represent plant community composition in the path model. Our model assumed that plant diversity could influence the relative amount of nitrification and denitrification by differential uptake of NH_4^+ and NO_3– by roots and associated microbiota (Ashton et al., 2010; Fig. 2 Path 1), indicated by square root transformed $\delta^{15}N\text{-}N_2O$. Plant community composition could also influence differential uptake of NH_4^+ and NO_3– by roots and associated microbiota because of varying functional traits (Fig. 2, Paths 2 and 3). Plant diversity (Fig. 2 Path 4) and plant community structure (Fig. 2 Paths 5 and 6) could also influence labile soil organic C (indicated by WEOC) by promoting decomposition through microenvironmental effects (Hector et al., 2000), which can then influence $\delta^{15}N\text{-}N_2O$ because labile C inputs can promote more incorporation of inorganic soil N into microbial biomass (immobilization), with preferential uptake of ^{14}N (Evans, 2007; Fig. 2 Path 7). Path 7 accounts for fractionation related to immobilization and relatively small fractionation occurs during N mineralization (gross mineralization = 0–5 ‰, N_2O production during nitrification = 0–70 ‰, N2O production during denitrification = 0–39‰; Evans, 2007), allowing paths 1–3 to be used as an indicator of effect on relative amounts of nitrification and denitrification. Log transformed-potential N_2O emissions could be influenced by the relative amount of nitrification to denitrification, because denitrification has long been considered the major source of N_2O (Knowles 1982; Fig. 2 Path 8). The final paths were a direct influence of plant diversity (Fig. 2 Path 9) and plant community structure (Fig. 2 Paths 10 and 11) on log transformed potential N_2O emissions, representing other mechanisms of plant diversity influence on N_2O that were not explicitly measured. The path diagram representing a test of our multivariate hypothesis was illustrated using webSEM (Zhang and Yuan 2012).

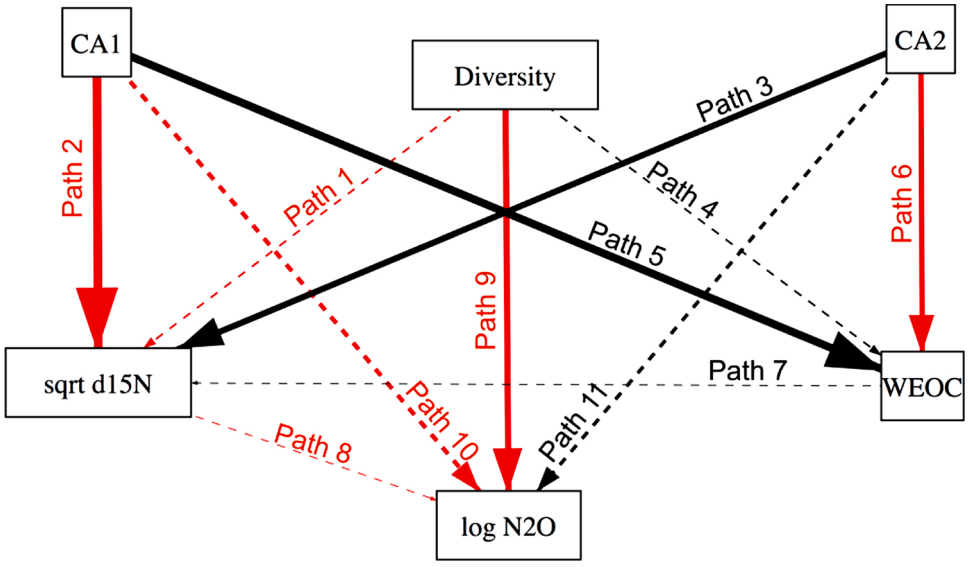

Fig. 2. Path model of mechanisms by which plant diversity (Shannon's diversity index) can influence potential nitrous oxide emissions. Line thickness corresponds with relative effect size (standardized path estimates in Table 2). Dotted lines represent non-significant paths. Positive effects are shown in black; negative effects are shown in red. Abbreviations: Diversity = plant Shannon's diversity levels (high [75th percentile and above] and low [25th percentile and below]), CA1 = first axis scores of correspondence analysis, CA2 = second axis scores of correspondence analysis, WEOC = water extractable organic carbon, sqrt d15 N = square root-transformed isotopic signature of N from nitrous oxide emissions, logN2O = log-transformed potential nitrous oxide emissions.

Potential mechanisms of plant diversity influence on potential N_2O emissions from soil were evaluated using a piecewise structural equation model (pSEM) with the piecewiseSEM package (Lefcheck 2006). Three site random intercept linear models fit with the nlme package were used in the pSEM: (1) plant diversity level and square root transformed $\delta^{15}N$-N_2O predict log transformed potential N_2O emissions (2) plant diversity level predict WEOC (3) WEOC and plant diversity level predict square root transformed $\delta^{15}N$-N_2O. Pseudo-R^2 values were reported as a comparative measure of fit for the endogenous variables in the pSEM, where marginal values represent a measure of fit based on only fixed effects and conditional values represent a measure of fit based on both fixed and random effects. Species richness and evenness were not included in the model because they were likely to be correlated with the plant diversity level predictor, based on Shannon Diversity Index values. Global-goodness-of-fit for the pSEM was assessed with tests of directed separation, where a non-significant P value indicates good model fit. This pSEM was also compared to a saturated pSEM, with an additional path of WEOC influencing log transformed potential N_2O emissions, by Akaike information criterion and Bayesian information criterion. Significant paths according to a type III test were evaluated and standardized path estimates were reported. Linear mixed models and pSEM omitted missing values. Estimated marginal means and associated standard errors for high and low diversity levels were calculated with the emmeans package.

3. Results

Sixty species were encountered in the final survey of all vegetation. The most frequently occurring species among all sites were *Sorghastrum nutans* ($n = 32$), *Coreopsis lanceolata* ($n = 29$), *Symphyotrichum ericoides* ($n = 29$), *Solidago missouriensis* ($n = 26$), *Monarda fistulosa* ($n = 22$), *Andropogon gerardii* ($n = 19$), *Oligoneuron rigidum* ($n = 18$), and *Solidago canadensis* ($n = 18$). The high diversity communities contained higher Shannon's diversity than the low diversity communities ($F_{1, 50} = 320.99$, $P < 0.001$; high diversity: 1.69 ± 0.06, low diversity: 0.73 ± 0.06). Diversity levels were associated with differences in Pielou's evenness ($F_{1, 50} = 105.73$, $P < 0.001$; high diversity: 0.91 ± 0.04, low diversity: 0.43± 0.04) and species richness ($F_{1, 27} = 7.95$, $P = 0.008$; high diversity: 7.21 ± 0.46, low diversity: 6.43 ± 0.46; replicate within site random effects used). Plant community composition (CA ordination) varied more with site than high or low diversity community type (Fig. 3). Soil pH ($F_{1, 50} = 0.09$, $P = 0.766$; high diversity: 5.72 ± 0.13, low-diversity: 5.75 ± 0.13; site-level averages provided in Table 1), extractable ammonium ($F_{1, 50} = 0.96$, $P = 0.331$; high diversity: 0.14 ± 0.04, low-diversity: 0.19 ± 0.04; site-level averages provided in Table 1), and extractable nitrate ($F_{1, 50} = 0.13$, $P = 0.721$; high diversity: 0.05 ± 0.01, low-diversity: 0.05 ± 0.01; site-level averages provided in Table 1) were not observed to differ between high and low-diversity levels.

Potential N_2O emissions were 6 times higher in communities with low plant diversity compared to the high diversity (Fig. 4 A). This pattern in estimated marginal means was associated with more zero-emission samples and fewer high-emission samples in high diversity plant communities compared to low diversity plant communities (Fig. 4 B). Potential N_2O emissions from soil were not related to abundance of the most frequently occurring species ($P > 0.05$), with the exception of *Coreopsis lanceolata* ($F_{1, 50} = 6.18$, $P = 0.016$), which was positively related to log transformed potential N_2O emissions (regression coefficient = 0.17). However, *Coreopsis lanceolata* percent cover was not observed to differ between plant diversity levels ($F_{1, 50} = 0.72$, $P = 0.401$; high diversity: 2.90 ± 1.15, low diversity: 4.22 ± 1.15).

The pSEM had a good overall fit ($P = 0.183$), with the following coefficients of determination: logN_2O (marginal = 0.16, conditional = 0.24), WEOC (marginal = 0.31, conditional = 0.42), and sqrtd^{15}N (marginal = 0.38, conditional = 0.38). The model indicated that plant diversity levels did not significantly influence relative amounts of denitrification and nitrification ($P = 0.907$, Table 2 Path 1). Plant community composition, however, had a negative effect on relative amounts of denitrification and nitrification with the first axis of CA scores ($P < 0.001$, Table 2 Path 2) and a positive effect with the second axis ($P = 0.004$, Table 2 Path 3). There was no significant influence of plant diversity level on WEOC ($P = 0.612$; Table 2 Path 4). Plant community composition, however, had a positive effect on WEOC in the first axis of CA scores ($P < 0.001$) and a negative effect with the second axis ($P = 0.006$). Water extractable organic carbon had no influence on $\delta^{15}N$ values ($P = 0.792$; Table 2 Path 7). There was also no significant difference in WEOC between diversity levels ($F_{1, 50} = 0.09$, $P = 0.612$; high diversity: 4.54 ± 0.24, low-diversity: 4.42 ± 0.24). Relative amount of nitrification compared to denitrification did not significantly influence potential N_2O emissions over one day (P = 0.340; Table 2 path 8). There was also no significant difference in isotopic signatures between diversity levels ($F_{1, 50} = 0.20$, $P = 0.659$; high diversity: 3.35 ± 0.44, low-diversity: 3.62 ± 0.44). Plant diversity level had a direct influence on potential N_2O emissions over one day, representing a mechanism or

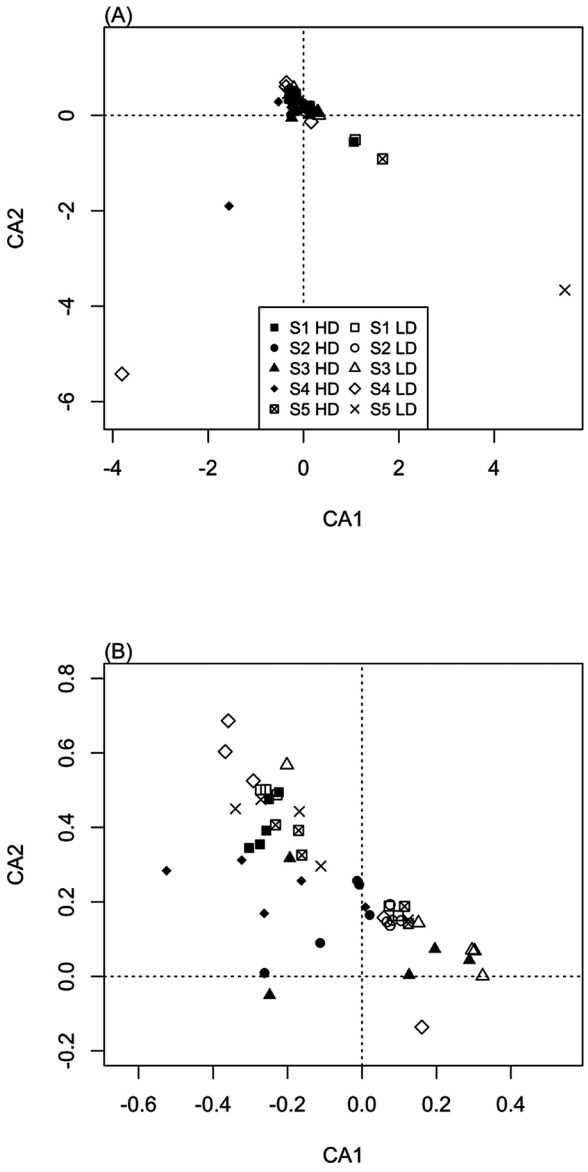

Fig. 3. Correspondence analysis based on percent cover values for each species. Panel A shows all points, while panel B shows the same ordination zoomed in on the cluster of points near the origin. Abbreviations: S = site; HD = high diversity; LD = low diversity. Site numbers correspond to descriptions in Table 1.

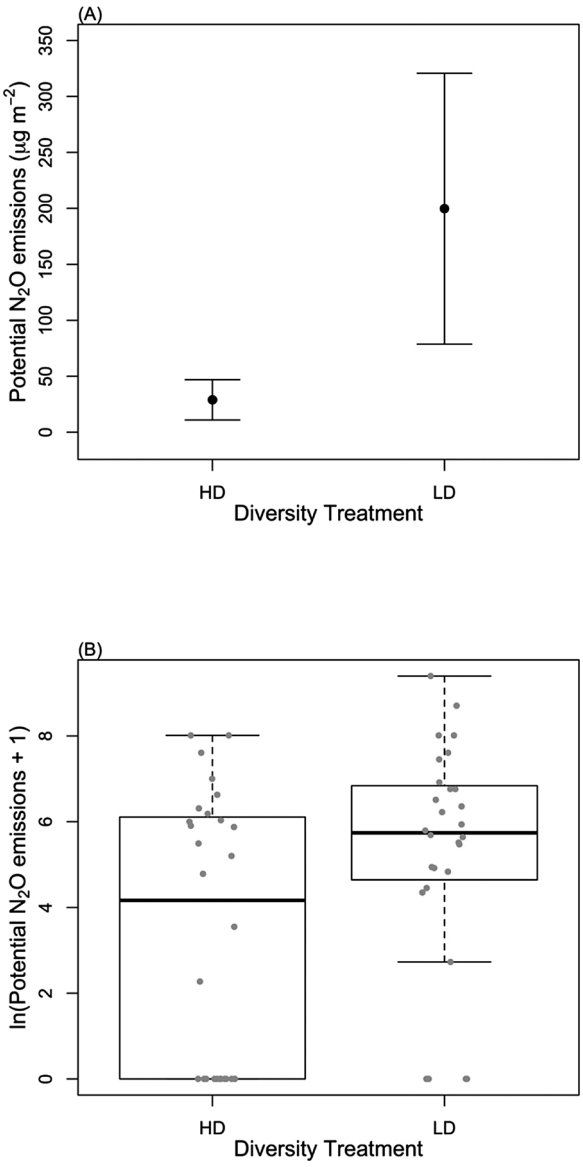

Fig. 4. (A) Estimated marginal means and associated standard errors for the direct influence of plant diversity level on potential nitrous oxide emissions measured over 1 day, back transformed from a ln(x+1) scale and (B) boxplot on the transformed scale. The high diversity level had a mean Shannon diversity index (H) value of 1.69 with a stand error of 0.045 and the low diversity level had a mean H value of 0.73 with a standard error of 0.031. Significance value is reported in Table 2.

combination of mechanisms not explicitly measured ($P = 0.012$; Table 2 path 9). In contrast, plant community structure did not have a significant direct influence on potential N_2O emissions (CA1: $P = 0.132$, CA2: $P = 0.161$).

4. Discussion

Greater N_2O emission from grassland restorations with low diversity plant communities is consistent with findings in multiple ecosystems (Ribas et al., 2015, Niklaus et al., 2016, Han et al., 2017, Piñeiro-Guerra et al., 2019). Our study was novel in that it considered a possible mechanism of plant diversity effect on potential N_2O emissions, specifically the influence of plant diversity on relative amounts of nitrification and denitrification via differential uptake of NH_4^+ and NO_3^- by plants and microbes. This mechanism was tested because greater nitrogen use with plant diversity has been demonstrated in several studies (Tilman et al., 2001, Ashton et al., 2010, Johnson et al., 2016), which could lead to less available N in high diversity plant communities and has been demonstrated in restored grassland (Klopf et al., 2017). This differential amounts of nitrification and denitrification mechanism, however, had little influence on potential N_2O emissions in grasslands restored from agriculture, suggesting an untested mechanism(s) is driving the plant diversity effect on potential N_2O emissions from soil. Despite the large difference in potential N_2O emission between the diversity levels, plant community structure had no direct or indirect effect on potential N_2O emissions, suggesting that plant alpha diversity is driving N_2O emissions from soil.

Relative availability of N and C can influence N_2O emissions from soil (Sotomayor and Rice 1995, Scott et al., 2019). The present study supported the finding that potential N_2O emissions from grassland restored from agriculture are limited by availability of inorganic N rather than organic C (Scott et al., 2019), because a model including labile C influence on potential N_2O emissions resulted in a worse model fit and inorganic nitrogen availability was low across all sites. Season can also influence N_2O emissions. Sampling was performed in the summer, when an average of 67% of annual N_2O emissions occur from unfertilized soybean fields in this region (Bremner et al., 1980). Our result that differential amounts of nitrification and denitrification, as would be expected if there were differences in plant uptake of N between high and low-diversity communities, had no effect on N_2O emissions from soil is consistent with the recent finding that belowground resource partitioning alone cannot explain ecosystem functions including plant nutrient uptake (Jesch et al., 2018). Progressive N limitation occurs during the first 10 years of grassland restoration (Baer et al., 2002; Rosenzweig et al., 2016) and likely limited the difference in extractable pools of N between high and low-diversity communities. Sites used in this study all were sown with more than 100 species and burned frequently. Klopf et al. (2017) used the same sites and found lower N availability in prairie restored and managed with more species compared to prairie sown with less than 10 species and rarely managed

Table 2
Significance values and standardized coefficients of paths from a piecewise structural equation model. Variable names and path numbers follow Fig. 2. Significant paths are presented in bold font.

Path	Response	Predictor	*P* value	Estimate	Standard Error	Standardized Estimate
1	sqrtd15 N	Diversity	0.907	−0.01	0.12	−0.02
2	**sqrtd15 N**	**CA1**	**< 0.001**	**-0.25**	**0.06**	**-0.54**
3	**sqrtd15 N**	**CA2**	**0.004**	**0.17**	**0.06**	**0.35**
4	WEOC	Diversity	0.612	0.13	0.25	0.07
5	**WEOC**	**CA1**	**< 0.001**	**0.46**	**0.11**	**0.47**
6	**WEOC**	**CA2**	**0.006**	**-0.31**	**0.11**	**-0.31**
7	sqrt d15 N	WEOC	0.792	0.02	0.06	0.04
8	log N2O	sqrt d15 N	0.340	0.81	0.88	0.12
9	**log N2O**	**Diversity**	**0.012**	**-1.90**	**0.77**	**-0.31**
10	log N2O	CA1	0.132	−0.73	0.47	−0.24
11	log N2O	CA2	0.161	0.61	0.43	0.20

by fire. Frequent burning likely contributed to low N availability, as frequent burning causes lower net N mineralization (Blair 1997).

Inclusion of site as a random effect explained additional variance, according to comparison of marginal and pseudo-R^2, for log-transformed potential N_2O emissions and WEOC. Explained variance in square root-transformed $\delta^{15}N$-N_2O, however, was not influenced by inclusion of site random effect. Site differences are reflected by differences in vegetation. For example, site 3 was characterized by weedy species (high abundance of red clover [*Trifolium pratense* L.] and the only site where common plantain [*Plantago major* L.] was observed). Sites 4 and 5 were located near a stream and were characterized by a high abundance of the sedge *Carex brevior* (Dewey) Mack.

This study highlights the need for studies that are spatially explicit and examine microbiomes of individual plant species. Abundance of one common species, *Coreopsis lanceolata*, was associated with potential N_2O emissions so comparative studies of soil microbiota associated with specific plant species might also improve N_2O emission predictions. However, the abundance of *Coreopsis lanceolata* did not differ with plant diversity levels, suggesting that the diversity effect is independent of the *Coreopsis lanceolata* effect on N_2O emissions from soil. Our results may appear to contrast the previous finding that plant identity rather than diversity mediates N_2O emissions from soils (Abalos et al., 2014), but this might be because the Abalos et al. study only considered grasses at two richness levels. Interestingly, the two components of diversity varied at different spatial scales; richness varied at replicate scale and evenness varied at site scale. Development of spatially explicit models might improve N_2O emission predictions. One recent spatially explicit study suggested that biological controls of N_2O emissions are strongest at large spatial scales (Piñeiro-Guerra et al., 2019).

The N_2O emissions we observed were low compared to another study with similar methods. Emission rate over 16 d from silt loam soils in grassland restorations (northeast Kansas, USA) was 0.36 g m^{-2} d^{-1} compared to our maximum value of 0.012 g m^{-2} from a 1 d incubation (Scott et al., 2019). We used a 40% water holding capacity (corresponding to ~60% water-filled pore space) so that nitrification and denitrification could both occur, and N_2O production would be maximized (Davidson 1991). The soil moisture levels ideal for N_2O production and low inorganic nitrate availability suggest N limitation. Further, this study is consistent with the tests of the Davidson model, which demonstrated sites dominated by ammonium rather than nitrate have lower N^2O production (Davidson et al., 2000).

We have ruled out differential amounts of nitrification and denitrification and deferential amounts of labile C inputs as mechanisms explaining the plant diversity effect on potential N_2O emissions. Further study is necessary to identify the predominant mechanism of plant diversity decreasing N_2O emissions. Candidate mechanisms include differences in microbial community composition or microbial physiology. A microbial composition effect could be examined with sequencing of functional genes, such as *amoA* (coding for ammonia monooxegenase, which catalyzes the rate limiting step of nitrification), *nosZ* (coding for nitrous oxide reductase, representing complete denitrifiers), and *nirS* and *nirK* (coding for nitrite reductase, representing denitrifiers that emit either N_2O or N_2). A microbial physiology effect could be examined with quantitative polymerase chain reaction of the same functional genes. Additional study is also needed to quantify the effect size of plant diversity on nitrous oxide emissions using *in situ* measurements over a wider diversity gradient and over a greater temporal range.

It is clear that managing for high-diversity plant communities has potential to reduce N_2O emission from soil, though the predominant mechanism remains elusive. Our study also indicates that high diversity plant communities are associated with less variability in N_2O emissions (Fig. 4). Greater variability may make modeling of N_2O emissions from soil with low diversity plant communities especially challenging. In addition to increasing biomass production (reviewed by Tilman et al., 2014, Chen et al., 2018), reducing NO_3– leaching (Tilman et al., 2001, Ashton et al., 2010, Johnson et al., 2016), faster aggregate formation (Klopf et al., 2017), and more C sequestration (Chen et al., 2018), less N_2O production from soil is an additional ecosystem service that further justifies restoration and management of ecosystems for plant diversity.

Data Accessibility

Data are provided as an electronic supplemental file.

Declaration of Competing Interest

The authors have no conflicts of interest to report.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pedobi.2020.150670.

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