DENITRIFICATION AND GAS EMISSIONS FROM ORGANIC SOILS UNDER DIFFERENT WATER TABLE AND FLOODING MANAGEMENT

J. O. Miller, P. G. Hunt, T. F. Ducey, B. S. Glaz

ABSTRACT. *Draining the Florida Everglades for agricultural use has led to land subsidence and increased phosphorus (P) loads to the southern Everglades, environmental concerns that can be limited by controlling water table depth. The resulting anaerobic conditions in saturated soils may lead to increased denitrification and gaseous N_2O release. Sugarcane was grown in lysimeters using four water table treatments, ranging from 40 to 16 cm in depth. Gas emissions were measured in April, July, and September using a photoacoustic gas analyzer. Denitrification enzyme activity (DEA) was performed on soil samples from the upper 15 cm, including non-limiting carbon and nitrogen conditions. No differences in N_2O emissions or DEA were observed between water table treatments. However, emissions of N_2O were higher in the spring, and CO_2 emissions were greater in the late summer. Overall, incomplete DEA was very low in the soil samples, indicating that shallow water tables will not increase N_2O emissions in these organic soils. Additions of C versus NO_3 increased DEA, indicating that these organic soils are carbon limited. Neither the* amoA *nor* nosZ *genes were affected by water table management, but the abundance of the* nosZ *gene increased from April to September, while* amoA *gene abundance decreased.*

Keywords. *Denitrification, N_2O, Organic soils.*

Prior to the construction of drainage canals, the Everglades were considered to be an impenetrable, seasonally flooded swamp (Davis and Ogden, 1994). Large-scale efforts to drain the Everglades region were started in 1907, creating an agricultural economy in central Florida (Davis and Ogden, 1994). Of the approximately 280,000 ha in the Everglades Agricultural Area (EAA), 155,000 ha are used for sugarcane production (Glaz and Morris, 2010). Due to the drainage of the EAA for agriculture, environmental concerns about P runoff and land subsidence have arisen (Daroub et al., 2009; Glaz et al., 2004; Rice et al., 2002). The subsidence occurring in the EAA is caused by microbial oxidation of organic matter, which was originally impeded by anaerobic conditions (Morris et al., 2004).

Best management practices were mandated in the 1990s to reduce P loads into the southern Everglades (Daroub et al., 2009). One of the practices to limit P movement was to control the amount of water discharged from each farm, leading to higher water tables within the EAA (Glaz and Morris, 2010; Glaz et al., 2004; Rice et al., 2002). These higher water tables, and the associated periodic flooding, can reduce sugarcane yields (Glaz and Morris, 2010).

The intermittent control and change of water table depths can also affect N cycling in the EAA. In Histosols, N cycling is affected by water table depth, which controls mineralization and denitrification (Terry, 1980; Terry and Tate, 1980). Originally, drainage of the EAA caused the aerobic breakdown of organic matter, leading to the mineralization of nitrogen (Terry, 1980). This inorganic nitrogen has not been observed in runoff, and one possible pathway may be denitrification (Terry, 1980; Terry et al., 1981). A comparison of potential denitrification between fallow, sugarcane, and grass soils observed three- to sevenfold greater denitrification enzyme activity (DEA) in the cropped fields (Terry and Tate, 1980). The greater DEA observed in cropped soils was attributed to decaying plant residues, providing the necessary carbon for denitrification (Hunt et al., 2007; Terry and Tate, 1980). By varying the water table depth over a growing season, denitrification and N_2O emissions can be affected by anaerobic conditions (Denmead et al., 2010; Glaz and Morris, 2010; Glaz et al., 2004).

Compared to mineral soils, a drained organic soil can produce a larger amount of N_2O (Terry et al., 1981), driven by plant residue (Rochette et al., 2010), nitrogen application (Dalal et al., 2003; Denmead et al., 2010), and water-filled pore space (WFPS) (Denmead et al., 2010). The frequent wetting of these organic soils, particularly when WFPS is in the range of 70% to 80%, controls N_2O flux (Denmead et al., 2010). Therefore, the adjustment of water table depth over a growing season will contribute to chang-

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es in DEA (Glaz and Morris, 2010; Glaz et al., 2004). Cropping systems can also affect N_2O flux; a Pahokee muck in Florida had greater N_2O flux from fallow fields, versus grass and sugarcane (Terry et al., 1981). The amount of N_2O from fallow fields was striking, since potential denitrification was greater in the cropped fields (Terry and Tate, 1980). Organic carbon was driving potential denitrification in the cropped fields, though, and the lack of carbon may have induced N_2O emissions in the fallow field (Terry and Tate, 1980; Terry et al., 1981).

Nitrite is an important component of denitrification, and Florida sugarcane soils have been found to have larger amounts of the ammonia-oxidizing bacterium *Nitrosomonas* than non-organic soils (Roesch et al., 2007). The conversion of ammonium into nitrite, via nitrification, and the greater C inputs of plant material may drive the DEA in EAA Histosols planted with sugarcane.

The current management practices used across the EAA may have an effect on DEA and greenhouse gas emissions. Because the areas are more prone to flooding and shallow water tables when drainage is limited, N_2O flux may also be greater in those soils with higher WFPS (Denmead et al., 2010; Glaz and Morris, 2010; Rice et al., 2002).

The bulk of global N_2O emissions have been determined to be biogenic in origin (Mosier et al., 1998). This biogenic production is the result of the microbial processes of nitrification and denitrification. The abundance of organisms responsible for performing these biological processes can be measured by quantitative real-time polymerase chain reaction (qPCR) using broad-range primers directed at the genes responsible for coding nitrification and denitrification enzymes (Chon et al., 2011; Ducey et al., 2011; Hallin et al., 2009). The nitrification-related gene *amoA* encodes the active site of ammonia monoxygenase, the enzyme responsible for oxidizing NH_3 into the intermediate hydroxylamine (NH_2OH). From this point, nitrous oxide originating from nitrification can potentially be produced via two pathways: first, via the oxidation of NH_2OH (Hooper and Terry, 1979) to nitrite; or second, via nitrifier denitrification (Wrage et al., 2001). In denitrification, N_2O is produced by the reduction of nitric oxide (NO) by the nitric oxide reductases. This N_2O, if it does not diffuse out of the microbial ecosystem and into the wider environment, can then be further reduced to N_2 by nitrous oxide reductase, encoded by *nosZ*.

The objectives of this study were to measure the effects of different water table depths and flooding in soil planted to sugarcane on (1) soil properties, (2) N_2O emissions, (3) DEA activity, and (4) the microbial populations involved in N_2O production.

Materials and Methods

Lysimeter Setup

Sixteen lysimeters were created by placing 1.8 m wide × 3.0 m long × 0.9 m depth fiberglass tanks into the ground at the USDA-ARS research station in Canal Point, Florida (Glaz and Morris, 2010; Glaz et al., 2004). Organic soil was collected in 1999 from local farms and placed into the lysimeters. Each lysimeter was filled with Pahokee muck soil (euic, hyperthermic Lithic Haprosaprist) and planted with four different sugarcane cultivars as split plots in 1/2 row per lysimeter. The cultivars included were CP 88-1762 (Tai et al., 1997), CP 89-2143 (Glaz et al., 2000), CP 89-2376 (Glaz et al., 2005), and CP 96-1252 (Edme et al., 2005). Water table depths were controlled using pumps, ball floats, and solenoids (Glaz et al., 2004) and were manually measured on most weekdays to ensure accuracy.

Table 1. Water table treatments by month, depth to water table, and presence or absence of flooding.

Treatment	Water Table Depth (cm)			Flooding
	May	July	August	
40a	40	40	40	No
4040	40	40	40	Yes
4016	40	40	16	Yes
1616	40	16	16	Yes

There were four water table treatments, replicated four times in the 16 lysimeters (table 1). In the first water table treatment, 40 always (40a), the water table was maintained at 40 cm depth. The 40a treatment was only flooded from 6 to 12 August 2011 for white grub (*Ligyrus subtropicus* Blatchley) control. The second water table treatment was flood-40-40-40 (4040), which was flooded cyclically from 22 June to 5 October 2010 for one week and then drained to a depth 40 cm for two weeks. The third treatment was flood-40-40-16 (4016), which was flooded under a similar schedule to 4040, but the drainage depth was changed from 40 to 16 cm from 24 August to 5 October 2010. The fourth water table treatment, flood-40-16-16 (1616), also had the same flooding cycle, but the drainage depth was 16 cm from 13 July to 5 October 2010.

Lysimeter Sampling

Field gas emissions were measured with a photoacoustic gas analyzer (PAGA; Innova 1412, LumaSense Technologies, Santa Clara, Cal.). A static chamber was inserted 5 cm into the soil, leaving a 1150 cm^3 headspace for gas sampling. Headspace gas was pumped into the PAGA every minute, and CO_2, N_2O, sulfur hexafluoride (SF_6), and water vapor were measured using the appropriate filter. Water vapor was used to correct N_2O concentrations measured by the PAGA, while manually injected SF_6 was measured to test the system for leaks.

The upper 15 cm of soil in the center of each lysimeter was analyzed on-site for electrical conductivity (EC), moisture, temperature, and oxygen reduction potential (ORP). Electrical conductivity and soil temperature were measured using a ECTestr11+ meter (Spectrum Technologies, East Plainfield, Ill.), while soil moisture was measured using a Delta-T HH2 moisture meter (Dynamax, Houston, Tex.). Oxygen reduction potential was measured using platinum and reference electrodes attached to a voltmeter. Soil samples for the measurement of DEA and microbial analysis were taken from the upper 15 cm of each lysimeter and placed on ice.

Laboratory DEA Analysis

DEA was measured by the acetylene inhibition method (Tiedje, 1994). For this analysis, soil (10 to 15 g) was

placed in 60 mL serum bottles (three bottles per sample per replication). The treatments (table 2) were as follows:

- DEAi: 5 mL of a solution containing chloramphenicol (1 g L^{-1}) to inhibit protein synthesis and to measure actual incomplete denitrification.
- DEA: 5 mL of a solution containing chloramphenicol (1 g L^{-1}) and 15×10^{-3} L of acetylene (produced from calcium carbide) to block denitrification at the nitrous oxide phase for measuring actual complete denitrification.
- DEAi/n: 5 mL of a solution containing chloramphenicol (1 g L^{-1}) and nitrates (200 mg L^{-1} NO_3-N) to measure potential incomplete denitrification.
- DEAn: 5 mL of a solution containing chloramphenicol (1 g L^{-1}) and nitrates (200 mg L^{-1} NO_3-N), and 15×10^{-3} L of acetylene to measure potential complete denitrification.

Four additional treatments were used to test for carbon limitations. These additional treatments were as follows:

- DEAi/g: 5 mL of a solution containing chloramphenicol (1 g L^{-1}) and glucose-C (600 mg L^{-1} glucose-C) to test for actual incomplete denitrification.
- DEAg: 5 mL of a solution containing chloramphenicol (1 g L^{-1}) and glucose-C (600 mg L^{-1} glucose-C), and 15×10^{-3} L of acetylene to test for potential complete denitrification.
- DEAi/gn: 5 mL of a solution containing chloramphenicol (1 g L^{-1}), glucose-C (600 mg L^{-1} glucose-C), and nitrates (200 mg L^{-1} NO_3-N) to test for actual incomplete denitrification.
- DEAgn: 5 mL of a solution containing chloramphenicol (1 g L^{-1}), glucose-C (600 mg L^{-1} glucose-C), nitrates (200 mg L^{-1} NO_3-N), and 15×10^{-3} L of acetylene to measure actual complete denitrification.

Serum bottles were capped with rubber septa, evacuated, and purged with purified N_2 gas three times. After purging, the appropriate serum bottles were injected with acetylene. Serum bottles were incubated on a horizontal shaker at 1.5 cycles s^{-1} and 24°C. After 3 h of incubation, 5 mL of the headspace gases were removed from the serum bottles with a syringe (Plastipak, Franklin Lakes, N.J.) and injected into vials (borosilicate glass, crimp top with butyl septum). The N_2O-N in the headspace gas was measured with a gas chromatograph (model 3800, Varian, Palo Alto, Cal.) equipped with a 15-mCi ^{63}Ni electron capture detector operating at 350°C. Chromatographic separation of the headspace gases was obtained by the use of 1.8 m long × 2 mm ID stainless steel column packed with Poropak Q (80-100 mesh, Alltech Associates, Deerfield, Ill.). Column and injector temperatures were 70°C, and the carrier gas was purified N_2. Samples were injected into the column by an auto-sampler (model 8200, Varian). All analyses were performed in triplicate.

Table 2. Denitrification enzyme assay (DEA) treatments by incomplete and total DEA.

Treatment	Type and Nutrient Additions
DEAi	Incomplete DEA
DEAi/n	Incomplete DEA with non-limiting NO_3 additions
DEAi/g	Incomplete DEA with non-limiting glucose additions
DEAi/gn	Incomplete DEA with non-limiting NO_3 and glucose
	With the Acetylene Block
DEA	Total DEA
DEAn	Total DEA with non-limiting NO_3 additions
DEAg	Total DEA with non-limiting glucose additions
DEAgn	Total DEA with non-limiting NO_3 and glucose

Soil Sample Analysis

Total C and total N were determined on air-dried soil samples using a TruSpec CN analyzer (Leco Corp., St. Joseph, Mich.). Water-soluble carbon was measured by placing 10 g of air-dried soil and 100 mL of DI water into a 250 mL flask. The slurry was shaken for 2 h, centrifuged at 5000 rpm, and filtered through a 1.5 µm glass filter. The filtrate was run on a liquid carbon analyzer (Shimadzu, Kyoto, Japan). To measure soil NO_3 and NO_2, a 2 M KCl extraction was mixed with 10 g air-dried soil in a 250 mL flask. The slurry was shaken for 1 h and then allowed to settle for 30 min. The slurry was then filtered with a 1.5 µm glass filter and analyzed on an autoanalyzer (Bran+Luebbe, Norderstedt, Germany).

Microbial DNA Extraction

Microbial DNA was extracted from all soil samples using a PowerLyzer Powersoil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, Cal.) according to manufacturer specifications. Final DNA quantity was determined via Biophotometer (Eppendorf, Hamburg, Germany), while quality was assessed by electrophoresis on a 1% agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, Cal.).

Quantitative Real-Time PCR Assays

All quantitative real-time PCR (qPCR) assays were performed using the LightCycler 480 Real-Time PCR System (Roche Diagnostics, Indianapolis, Ind.). Abundances of ammonia monooxygenase subunit A (*amoA*) and nitrous oxide reductase (*nosZ*) in soil were measured using primers synthesized by Integrated DNA Technologies (Coralville, Iowa). Fragments of *amoA* and *nosZ* were amplified via qPCR using primer pairs *amoA*-1F (Rotthauwe et al., 1997) and amoAr NEW (Hornek et al., 2006), and nosZF and nosZ-1622R (Throback et al., 2004), respectively. Assays were carried out using SYBR GreenER qPCR SuperMix (Invitrogen, Carlsbad, Cal.) in a total volume of 25 µL. The final reaction concentration of reagents was as follows: 1× SYBR GreenER qPCR SuperMix, 200 nM each of forward and reverse primers, and 10 ng of DNA template. The qPCR reaction conditions were as follows: (1) initial denaturation at 95°C for 5 min, (2) 50 cycles of denaturation at 95°C for 30 s, an annealing temperature of 54°C (*amoA*) or 55°C (*nosZ*) for 30 s and elongation at 72°C for 30 s, and (3) melting curve analysis to confirm amplification product specificity. Fluorescent measurements were taken during the annealing phase of each cycle. Data were collected and processed using the LightCycler 480 software package. All qPCR assays included control reactions without template. Each assay also contained appropriate standard DNA reactions with concentrations between 10^1 and 10^9 copies per reaction, which were used to calculate amplification efficiencies according to the equation: $E = 10^{[-1/slope]}$ (Pfaffl,

2001). DNA standards consisted of plasmids carrying the appropriate target gene, which were previously sequenced to confirm their identity and primer binding sites (Ducey et al., 2011). Each assay was performed in triplicate, with duplicate measurements for each sample. Prior to statistical analysis, *amoA* and *nosZ* gene abundances were normalized to the amount of DNA used per assay (ng^{-1}) and log_{10} transformed. Gene abundances were also corrected for copies per organism, with one copy per organism for the denitrification gene *nosZ* (Kandeler et al., 2006) and 2.5 copies per organism for *amoA* (Chain et al., 2003).

Statistical Analyses

Analyses of DEA, soil properties, and emissions were done as a randomized complete block design, using the PROC GLIMMIX procedure in SAS (SAS Institute, Inc., Cary, N.C.). Correlations of soil properties, DEA, and gas emission were done using the PROC CORR procedure in SAS. Correlations between gene abundances and environmental variables from each month's treatment schedule were calculated using the Analyse-it plug-in (Analyse-it Software, Ltd., Leeds, U.K.) for Excel 2007 (Microsoft Corp., Redmond, Wash.).

Results and Discussion

Soil Properties

Soil properties varied among the four water table treatments (tables 1 and 3), and this variation was related to both flooding events and depth to water. The 4016 and 1616 treatments had the highest moisture, measuring 49.0% and 48.2%, respectively. This higher moisture was related to their shallower water tables (Morris et al., 2004). Similarly, in the 4016 and 1616 treatments, the oxygen reduction potential (ORP) was lowest. In the 40a treatments, water-soluble carbon (WSC), pH, and electrical conductivity (EC) were all greatest (table 3). The 40a treatments were not flooded through the summer, and their greater salt and carbon contents may reflect lower leaching. Previously, it has been reported that flooding organic soils releases soluble carbon (Reddy, 1982), possibly due to release of WSC from sugarcane roots (Morris et al., 2004). Although the 4016 and 1616 plots had greater moisture, the leaching of these plots may have countered WSC release. Release of NO_3 following rainfall on organic soils has also been observed (Terry and Tate, 1980), but no difference was observed in NO_3 content among the water table treatments (table 3). Soil NO_3 can also be removed by crop uptake in organic soils and leaching, just as in mineral-based soils (Rochette et al., 2010; Southwick et al., 1995; Terry and Tate, 1980). However, in organic soils, mineralization is often faster than crop uptake (Reddy, 1982). Thus, it is reasonable to assume that rapid mineralization and nitrification was occurring in these organic soils (Reddy, 1982; Rochette et al., 2010; Terry, 1980).

When averaged by month, WSC was lowest in September, possibly due to the leaching of treatment lysimeters over the summer (table 3). The C:N ratio was also lowest in September, but this was due to the greater NO_3 contents (table 3). The decrease in NO_3 from April to July may indicate depletion by plant uptake, followed by mineralization of organic matter and more limited plant uptake in September (Reddy, 1982; Rochette et al., 2010). The release of NO_3 has also been reported to increase with average daily temperature (Reddy, 1982); this is consistent with the NO_3 increases observed from April to September (3.5 and 5.0 mg L^{-1}, respectively).

Field Gas Emissions

In organic soils, greater N_2O emissions were previously observed at 40 versus 80 cm water table depths (Berglund and Berglund, 2011). In general, N_2O production is tied to shallow water tables and higher soil moisture content (Adviento-Borbe et al., 2006; Flessa et al., 1998; Rochette et al., 2010). In this study, there were no observable effects between 16 and 40 cm water table depths (table 3). Relative to the time of the growing season, greater N_2O emissions were observed in April (table 3). This finding is similar to that found in drained organic soils in Sweden (Berglund and Berglund, 2011; Flessa et al., 1998). The lack of N_2O emissions in July and September may have been due to the high pH (Flessa et al., 1998), but the shift in pH was not that great. Previously, it has been reported that greater moisture and lower C:N ratio can produce more N_2O emissions (Berglund and Berglund, 2011; Flessa et al., 1998; Hunt et al., 2007; Klemedtsson et al., 2005; Rochette et al., 2010). In the organic soils observed in this study, the C:N ratio may not be a dominant factor in influencing N_2O emissions.

In contrast to N_2O, the CO_2 emissions were highest (34 g CO_2 m^{-2} d^{-1}) in the 4040 treatments (table 3). While

Table 3. Soil physical and chemical properties measured in field and laboratory settings averaged by treatment and month.

Treatment[a] and Month	Moisture (%)	ORP (mV)	pH	EC (µS cm^{-1})	C:N Ratio	WSC (mg L^{-1})	NO_3 (mg L^{-1})	CO_2 (g m^{-2} d^{-1})	N_2O (mg m^{-2} d^{-1})
40a	46.5 bc	197 ab	8.3 a	1913 a	17.8	100.2 a	3.8	27 b	2.6
4040	45.6 c	205 a	8 b	1509 b	17.8	59.1 b	3.9	34 a	2.6
4016	49 a	128 c	8 b	1540 b	18.7	57.5 b	3.1	26.7 b	2.7
1616	48.2 ab	157 bc	7.9 b	1632 ab	18	60 b	4	25.7 b	2.3
LSD[b]	**	**	***	*		***			
April	43.3 c	-39 c	7.7 b	2157 a	18.5 b	77.4 a	3.5 b	24 b	6.6 a
July	46.4 b	334 a	8.4 a	1093 c	20.1 a	66.9 ab	2.6 c	31.7 a	0.7 b
September	52.2 a	219 b	8 c	1696 b	15.5 c	62.9 b	5.0 a	29.4 ab	0.4 b
LSD[b]	***	***	***	***	***	*	***	*	***

[a] 40a = water table at 40 cm not flooded, 4040 = water table at 40 cm, 4016 = water table at 16 cm in August, 1616 = water table at 16 cm in July, and WSC = water-soluble carbon.

[b] LSD ($\alpha = 0.05$): * = 0.05, ** = 0.01, and *** = 0.001.

high CO_2 emissions in deeper water tables, similar to the 4040 treatments, have previously been observed to be greater (Moore and Knowles, 1989), the 40a treatments had the same water table depth but lower CO_2 emissions. When compared to 40a treatments, cyclical flooding of the 4040 treatments probably caused the increased CO_2 emissions from these lysimeters due to wetting and drying cycles (Morris et al., 2004). Carbon dioxide emissions can be inversely related to EC (Adviento-Borbe et al., 2006); in July, the greatest CO_2 emissions coincided with the lowest EC measured (table 3). Similarly, low emissions of CO_2 have previously been observed in the spring and greater in July in organic soils in Sweden (Berglund and Berglund, 2011). It has also been reported that CO_2 emissions decrease with greater matric tension (Berglund and Berglund, 2011), so the wetter soils in July and September may have also increased CO_2 emissions.

Denitrification Enzyme Activity

When DEA treatments (table 2) were averaged by month and water table treatment, the incomplete DEA treatment receiving both NO_3 and glucose (DEAi/gn) was the greatest (fig. 1a), followed by glucose additions (DEAi/g), and the control (DEAi). Incomplete DEA with non-limiting NO_3 additions (DEAi/n) was similar to both DEAi/g and DEAi (fig. 1a). Similar results were seen for total DEA, except that DEAn and the control (DEA) were similar to each other (fig. 1b). This documents that for both incomplete (fig. 1a) and total (fig. 1b) DEA, carbon was the most limiting factor in these organic soils. If the flooded plots contain soluble carbon, it does not appear to have been readily available to denitrifying microbes (fig. 1).

Relative to the growing season, there was no difference in DEA between the four water table treatments, except for incomplete DEA in September (fig. 2). In September, the 40a and 4040 water table treatments had greater DEAi/g (25.3 and 24.5 µg N kg soil^{-1} h^{-1}, respectively) than the shallow water table treatments (4016 and 1616). This is similar to organic soils drained at different depths, where soils with shallower water tables had greater gas emissions (Berglund and Berglund, 2011). Water-soluble carbon content (table 3) does not seem to be a factor in denitrification (i.e., glucose additions); it was higher in the 40a treatments (100.2 mg C L^{-1}) but not in the 4040 treatments (59.1 mg C L^{-1}).

By month, some differences were observed in DEA (fig. 3). April had greater incomplete DEA (fig. 3a) in the DEAi (12 µg N kg soil^{-1} h^{-1}) and DEAi/g (56.8 µg N kg soil^{-1} h^{-1}) treatments. The greater DEAi observed in April coincided with higher field N_2O emissions (table 3) as well. The C:N ratio was lower than July (table 3), so the addition of carbon in the form of glucose should have produced lower amounts of incomplete DEA (Berglund and Berglund, 2011; Hunt et al., 2007). Water-soluble carbon was also the highest in April (table 3), so adequate carbon should have been available for complete denitrification. Therefore, the extracted WSC may not have been contributing to denitrification in these soils. Nitrate is released from peat soils when C:N < 20 (Klemedtsson et al., 2005),

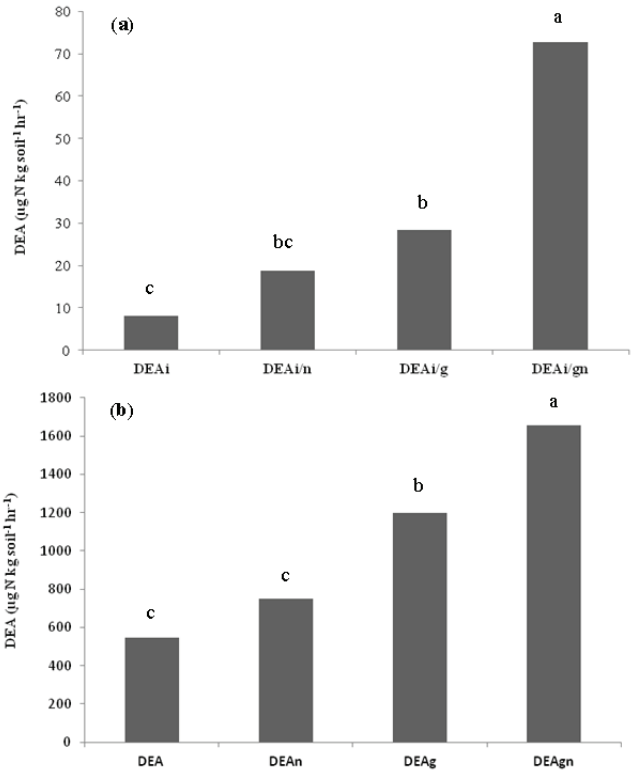

Figure 1. Average effect of nitrate and glucose on (a) incomplete DEA, and (b) total DEA on Florida sugarcane soils (i = incomplete, n = nitrate, and g = glucose). Letters represent statistical differences by LSD (α = 0.05).

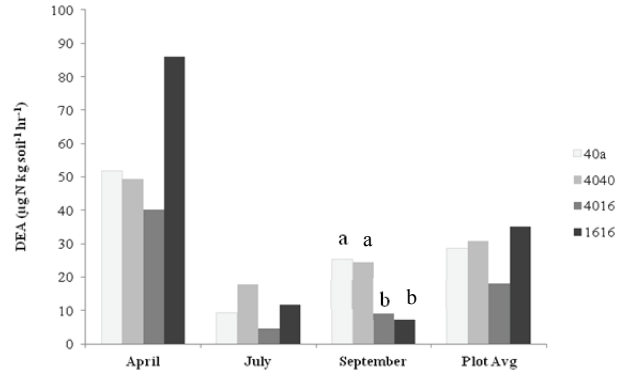

Figure 2. Incomplete DEA with glucose additions compared between water table treatments for each month. Letters represent statistical differences by LSD (α = 0.05).

which may explain why NO_3 was less limiting in these soils (table 3).

For total denitrification (fig. 3), April had the lowest DEA, DEAn, and DEAgn. This may be related to lower microbial and enzyme activity in the spring months. No differences for DEAg were observed by month, although WSC dropped over the summer. Again, this seems to indicate that WSC did not influence DEA in these soils. In September, greater DEAn was observed (fig. 3), along with the highest NO_3 contents (table 3). The higher NO_3 observed in September may have coincided with increased enzyme activity, so that additional NO_3 was readily denitrified. The

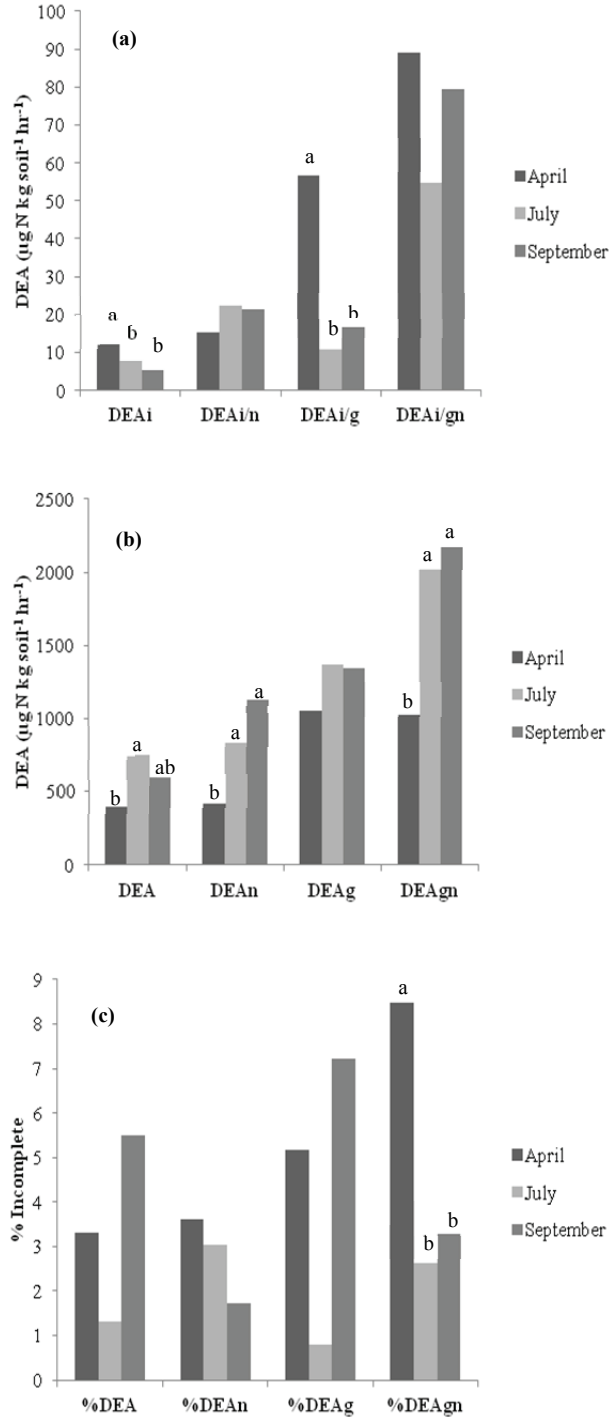

Figure 3. Average DEA by month and treatment for (a) incomplete DEA, (b) total DEA, and (c) % incomplete DEA (i = incomplete, n = nitrate, and g = glucose). Letters represent statistical differences by LSD (α = 0.05).

higher moisture in September (table 3) may have also increased enzyme activity.

In April, the 4016 and 1616 plots responded negatively to the addition of NO_3 relative to the control (data not shown). This changed in July, when the 40a plots responded negatively. Nitrate should not be limiting in these peat soils (Klemedtsson et al., 2005), and N_2O emission response has been lower in non-irrigated soils receiving nitrogen fertilizer (Rochette et al., 2010).

Percent incomplete denitrification (fig. 3) was calculated by dividing incomplete DEA by total DEA for each non-limiting treatment. For these organic soils, percent incomplete DEA was less than 10% in all treatments (fig. 3c). This is in contrast to organic soils in North Carolina, which have percent incomplete DEA greater than 50% (unpublished data), and Coastal Plain riparian buffers, which have percent incomplete DEA from 25% to 45% (Hunt et al., 2007). Thus, most of the denitrification occurring in these organic soils is going to completion. The percent incomplete DEA in April was greater for DEAgn, which means that where carbon and nitrogen were not limiting, more DEA was incomplete (fig. 3c). Nitrogen should be the most limiting factor in Histosols (Klemedtsson et al., 2005), but it appears that fresh carbon is also important in these EAA soils (Rochette et al., 2010).

MICROBIAL ANALYSIS

Temporally, the patterns between *amoA* and *nosZ* gene abundances were the opposite (table 4). From April to September, *amoA* gene abundances decreased over 55% across all treatments, whereas, with the exception of 4016, which remained static, *nosZ* gene abundances increased over 30% during this period. Overall, gene abundances of *amoA* were comparable to levels found in tidal wetlands; however, *nosZ* levels were two orders of magnitude higher in these soils than the tidal wetlands (Bannert et al., 2011). Likewise, *nosZ* levels were two orders of magnitude higher in our organic soils compared with constructed wetlands (Chon et al., 2011). These high *nosZ* levels may be related to the low N_2O emissions measured in the soils, as well as the low levels of incomplete DEA recorded in this study.

The relationships between soil properties and abundances of both *amoA* and *nosZ* genes were examined by regression analysis. For *amoA*, a significant, negative relationship was observed with total N ($r = -0.31$, $p = 0.03$) and NO_3 ($r = -0.31$, $p = 0.005$). The relationship of increasing *amoA* abundances to decreasing total N levels could coincide with the removal of NH_3 from the environment by active ammonia oxidizing populations. Likewise, the relationship of increasing *amoA* abundance to decreasing NO_3 levels may be attributed to the close association of ammonia oxidizing organisms with organisms that utilize nitrate. A similar relationship has been previously reported (Gubry-Rangin et al., 2010). For the *nosZ* gene, significant positive relationships were observed with total N ($r = 0.35$, $p = 0.02$) and NO_3 ($r = 0.33$, $p = 0.01$). Since NO_3 feeds directly into the denitrification cycle, this observation may be indicative of an increase of overall denitrifier abundances in response to available nitrogen resources.

There was a significant positive relationship between *amoA* gene abundances and N_2O emissions ($r = 0.52$, $p < 0.001$), a result that may be indicative of N_2O emissions from nitrifier-related hydroxylamine oxidation or nitrifier denitrification. Another explanation is that products downstream of the nitrification process, such as NO_3, were subsequently made available for the heterotrophic population

Table 4. Copy numbers of *amoA* and *nosZ* genes as determined by qPCR by treatment and month.

Gene[a]	Treatment	Gene Copy Number per ng of Extracted DNA[b]		
		April	July	September
amoA	1616	3.1×10^3 (3.8×10^2)	4.1×10^3 (4.8×10^2)	1.2×10^3 (9.4×10^1)
	4016	3.6×10^3 (5.5×10^2)	1.6×10^3 (1.4×10^2)	1.5×10^3 (1.1×10^2)
	4040	3.6×10^3 (3.6×10^2)	2.5×10^3 (3.6×10^2)	1.5×10^3 (8.7×10^1)
	40a	3.2×10^3 (3.9×10^2)	3.5×10^3 (3.9×10^2)	1.5×10^3 (1.5×10^2)
nosZ	1616	3.6×10^5 (2.9×10^4)	4.8×10^5 (5.3×10^4)	4.8×10^5 (3.6×10^4)
	4016	2.0×10^5 (9.2×10^3)	2.1×10^5 (2.0×10^4)	2.1×10^5 (2.9×10^4)
	4040	2.4×10^5 (2.8×10^4)	2.1×10^5 (1.8×10^4)	4.2×10^5 (3.6×10^4)
	40a	2.4×10^5 (2.0×10^4)	3.4×10^5 (2.8×10^4)	4.3×10^5 (2.9×10^4)

[a] PCR efficiencies were as follows: *amoA* (slope = -3.69, $r^2 = 0.99$, E = 1.87); *nosZ* (slope = -3.39, $r^2 = 0.95$, E = 1.97).
[b] Values are means (and standard errors).

to perform incomplete denitrification, especially in the month of April when *amoA* and N_2O emissions were at their highest. This postulation is supported by gene abundances of *nosZ*, which show a significant negative relationship to N_2O emission rates (r = -0.39, p = 0.005), showing the highest *nosZ* abundances and lowest N_2O emission rates in September. Neither *amoA* nor *nosZ* gene abundances had a significant relationship with denitrification enzyme activity rates.

CONCLUSIONS

Cyclical flooding of organic soils in the EAA caused leaching of both WSC and salt from the upper 15 cm of the soil. Greater moisture was observed with shallow water tables. We detected no effect of water tables on N_2O emissions from these organic soils. However, greater N_2O emissions occurred in the spring compared with the summer, possibly due to fresh NO_3 content or greater *amoA* gene abundances, which may have been an indicator of hydroxylamine oxidation or nitrifier denitrification.

No differences in DEA among water table treatments were observed. Overall, N_2O from incomplete DEA was low for these soils; this result was not expected due to the low C:N ratios. Additions of glucose had a greater effect than NO_3 on DEA, which indicates that fresh, soluble carbon is important to DEA in these organic soils.

Gene abundances for *amoA* decreased from April to September, which may have been due to the loss of ammonia in these soils. The increased abundances of the *nosZ* gene from April to September were potentially related to an increase in NO_3. The increase in *nosZ* gene abundances is consistent with the lower incomplete DEA and N_2O emission rates in September. Overall, water table management had no substantial effects on the emissions of N_2O from these typical organic soils of the Florida EAA.

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