Geoderma 245–246 (2015) 74–82

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Patterns and controls of anaerobic soil respiration and methanogenesis following extreme restoration of calcareous subtropical wetlands

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ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 23 January 2015
Accepted 27 January 2015
Available online 3 February 2015

Keywords:
Carbon
CO_2
Everglades
Marl
CH_4
Phosphorus

ABSTRACT

Wetland restoration is globally important to reestablish functions such as carbon (C) sequestration; however, restoration activities (e.g., land clearing) can affect anaerobic C cycling and greenhouse gas production. In this study, we compared enzyme activity (ß-glucosidase) and anaerobic production of carbon dioxide (CO_2) and methane (CH_4) in soils of reference wetlands and those restored (in 2000 and 2003) by complete soil removal (CSR) after farming in an area of Everglades National Park. We found elevated gaseous production and enzyme activity in restored relative to reference wetlands. Correlations with measured activities suggest soil phosphorus (P) and C content explain site differences in C processing. We tested the potential for P and labile C limitation through direct additions of P and glucose. Production of CO_2 and CH_4 in both systems was stimulated by glucose; however, P only stimulated CH_4 production in reference wetlands. This suggests that decomposition is limited by labile C regardless of restoration, and that soil P concentrations potentially regulate CH_4 production. Additional studies are necessary to establish CH_4 production pathways and investigate the interaction between P and C availability. Following CSR, wetlands are hypothesized to shift from nitrogen (N) to P limitation; therefore, CH_4 production may decrease with restoration age while approaching P limitation.

1. Introduction

Wetlands have decreased by approximately 50% worldwide (Zedler and Kercher, 2005). Subsequently, restoration efforts have increased to reestablish essential wetland functions and achieve a "no net loss" standard. An assortment of restoration efforts have been tested and vary depending on site characteristics and restoration goals. These approaches range from less invasive techniques such as manipulating vegetation (Andersen et al., 2006; Smith et al., 2011), to more intensive techniques such as restoring hydrology (Acreman et al., 2007) or ecosystem maintenance through prescribed fire (Doren et al., 1991; Hamman et al., 2008). Wetlands which have accumulated excess nutrients, such as agricultural lands, are commonly targeted for mitigation (Aldous et al., 2007; Van Dijk et al., 2004). One of the restoration techniques to remove excess nutrients is topsoil removal (Klimkowska et al., 2007; Ross et al., 1982; Tallowin and Smith, 2001; Verhagen et al., 2001) which can include the extreme condition of complete soil removal (CSR) to bedrock (Dalrymple et al., 2003).

Soil disturbance following this restoration technique can have drastic effects on ecosystem function. Changes in vegetation (composition and abundance) or organic matter (OM) structure during soil disturbance may disrupt the ecosystem C balance (source/sink) (Höper et al., 2008; Kimmel and Mander, 2010) altering greenhouse gas (CO_2 and CH_4) emissions (Andersen et al., 2006; Bortoluzzi et al., 2006; Jauhiainen et al., 2008). With soil nutrient availability as a known control of CH_4 production in some ecosystems (Smith et al., 2007; Wright and Reddy, 2001) changes in nutrient content following soil disturbance should be evaluated.

Extensive research has been conducted and is ongoing in the greater Everglades (Florida, U.S.A.) to restore ecological function following alteration of hydrology and agricultural nutrient enrichment (Richardson, 2008). However, within Everglades National Park, the extreme restoration technique of complete soil removal is being tested in the Hole-in-the-Donut (HID) region as an attempt to remove and discourage regrowth of invasive vegetation (Dalrymple et al., 2003). The HID is a collection of calcareous marl prairie wetlands which were heavily farmed from the early 1900s through 1975 (Smith et al., 2011). Continuous fertilization and soil disturbance following the use of mechanical farming equipment altered the soil aeration status and structure while drastically increasing soil nutrient concentrations. Once farming ceased, these lands were invaded by *Schinus terebinthifolius* Raddi and vegetation typical of the oligotrophic Everglades such as *Cladium* ceased to exist (as described by Ewel et al., 1982). Sites were mechanically-cleared as an extreme restoration approach and were allowed to re-vegetate naturally and soils which were high in available P are now hypothesized to shift from nitrogen (N) to P limitation while approaching the native/reference status (Inglett et al., 2011; Liao et al., 2014).

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http://dx.doi.org/10.1016/j.geoderma.2015.01.018
0016-7061/

Previous work by Smith et al. (2007) investigated CH_4 production following CSR in the HID, concluding that restored sites were elevated in CH_4 production compared to those of undisturbed reference areas. Regulators of CH_4 production including nutrient limitation warrants further investigation. In wetland soils, methanogens rely on simple substrates released from respiring or fermenting microbes (Conrad, 1999), a process which is often controlled by enzyme activity and the degradation of complex OM (Sinsabaugh et al., 1993). However, an additional control on microbial decomposition can be a nutrient limitation in some ecosystems (Hogg et al., 1994). Increasing P concentrations have been linked to elevated CH_4 production (Pivničková et al., 2010) although this is not always the case (Bridgham and Richardson, 1992; Drake et al., 1996) suggesting the relationship between these parameters is complex and may depend on initial soil P concentrations and OM quality.

The potential coupling of P biogeochemistry and C dynamics has important implications for wetland restoration and the subsequent ability for newly restored wetlands to function as a sink/source of C greenhouse gases. Wetlands are the largest natural source of CH_4 (Lelieveld et al., 1998), with tropical and southern wetlands accounting for more than 70% of this CH_4 source (Solomon et al., 2007). Therefore, understanding how restoration can affect the C balance and subsequent greenhouse gas release from these ecosystems is crucial. For this reason, we conducted the following study to investigate the patterns of anaerobic greenhouse gas production (CO_2 and CH_4) following the extreme restoration technique of complete soil removal.

Following restoration, soils were enriched in P and organic carbon (OC) relative to the unamended reference soil. Thus, our objective was to investigate the relationship between soil nutrients (P and C) and anaerobic C gas production (CO_2 and CH_4). To determine if differences in microbial activity between restored and reference soils were related to anaerobic C gas production we investigated the importance of microbial biomass carbon (MBC) and β-glucosidase enzyme activity (as a source of available non-methanogenic substrates) as a predictor of site C gas production. In addition, we assessed the relationship between greenhouse gas production and nutrient limitation in both unamended soils and those incubated with C or P in two separate microcosm experiments. We hypothesized that CO_2 and CH_4 production would be elevated in restored relative to reference soils and elevated P (decreased microbial nutrient limitation) would correspond with heightened C processing.

2. Methods

2.1. Site description

The Hole-in-the-Donut (HID) region of Everglades National Park (Miami Dade County, Florida, U.S.A.) consists of abandoned crop fields overlying a marl prairie wetland ecosystem (Fig. 1). These soils are Entisols which consist of Biscayne and Perrine marl with poor to poorly drained characteristics (USDA, 1996). Restoration of this area began in 1989 with mechanical removal of invasive vegetation followed by complete soil removal to bedrock (Dalrymple et al., 2003; Inglett and Inglett, 2013). In the current study, plots restored in years 2000 (Res00) and 2003 (Res03) were compared to a reference (never farmed) marl prairie wetland located within the HID vicinity. Based on restoration time and prior fertilization history each site varied in biogeochemical properties including soil nutrients, soil depth, and microbial parameters (Table 1). Vegetation in the reference site is typical of oligotrophic Everglades dominated by *Cladium jamaicense* Crantz (Loveless, 1959) with *Muhlenbergia capillaris*, *Andropogon* and *Schoenus* spp. In contrast, restored sites are dominated by a mixture of invasive and undesirable vegetation including *Baccharis halimifolia* and to a lesser extent *Ludwigia* spp., *Salix* sp. and *Typha domingenesis*.

2.2. Soil sampling

To encompass elevation variability, five approximately equidistant stations were selected along a 1.5 km transect within each of Res00, Res03, and reference wetlands (Fig. 1, Liao and Inglett, 2012). Soil depth at each site was measured with a soil probe (30 total readings per sampling location, Table 1). In October 2009 (wet season), three composite samples (5 grabs each) from 0 to 5 cm depth (or until bedrock) were collected using a spatula from each transect sampling location (15 composite samples per site). To estimate bulk density additional samples ($n = 3$) were taken using a sharpened steel tube. Soils were placed on ice and returned to the University of Florida for processing within two days of collection.

Soil samples were sieved through a 2 mm mesh to remove rocks and roots. Representative aliquots of field-moist soil were removed from each sample and analyzed for multiple microbial properties including microbial biomass carbon (MBC), MBN and MBP, BGA, anaerobic respiration (CO_2) and methanogenic potentials (CH_4). Soil moisture content averaged 54 and 46% for restored and reference wetlands, respectively. Separate soil aliquots were dried at 105 °C for three days, hand ground with a mortar and pestle and used for determination of total carbon, nitrogen, and phosphorus (TC/TN/TP).

2.3. Soil nutrients and microbial biomass analyses

Soil nutrient analyses were conducted by the Wetland Biogeochemistry Laboratory at the University of Florida (Gainesville, FL). Microbial biomass C/N/P was analyzed by extraction following chloroform fumigation (Vance et al., 1987). Briefly, soil samples were extracted with 0.5 M K_2SO_4 (MBC and N) or 0.5 M $NaHCO_3$ (MBP) after being incubated with (fumigated) and without (control) chloroform. Filtered (0.2 μm) extracts were analyzed for total extractable organic carbon (TOC) using a 5050A TOC auto-analyzer (Shimadzu Corp., Columbia, MD; EPA method 415.1). The difference in extractable TOC between the fumigated and non-fumigated samples was considered MBC following correction with an extraction efficiency (K_{EF}) of 0.37 (Sparling et al., 1990). For MBN determination, extracts were analyzed following Kjeldahl digestion on a Technicon™ auto-analyzer (EPA method 351.2). The difference between fumigated and non-fumigated TKN was considered MBN following correction with an extraction efficiency (K_{EF}) of 0.54 (Brookes et al., 1985). Extracts were analyzed on a Technicon™ auto-analyzer (EPA method 365.1).

Soil TN and TC were analyzed using a Thermo FlashEA 1112 series NC soil analyzer (Thermo Fisher Scientific, Inc., Waltham, MA). Loss on ignition (% LOI) of soil samples was determined after combustion at 550 °C for 3–4 h. Soil OC was estimated using LOI assuming 45% OC content factor derived from total OM (Wright et al., 2008). Soil TP was determined following sequential combustion at 550 °C for a 4 hour period and dissolution of remaining ash with 6 M HCl (Andersen, 1976). Extracts were analyzed colorimetrically for reactive P using a Technicon™ Autoanalyzer III (SEAL Analytical, Mequon, WI) (EPA method 365.1). Extractable inorganic P (P_i) was determined in soil samples using 0.5 M $NaHCO_3$ according to method 365.1 (USEPA) (Kuo, 1996).

2.4. Anaerobic respiration and methanogenesis

Anaerobic CO_2 and CH_4 potentials were quantified during laboratory incubations. Unfortunately, due to shallow soil depth in restored sites, field gas measurements (flux) were unfeasible. Approximately 2 g dry weight soil and 10 mL of nanopure water (treated with UV) were combined in 30 mL serum tubes ($n = 15$ per site), sealed with butyl rubber stoppers and aluminum crimp tops, flushed with N_2 for 15 min to encourage anaerobic conditions and stored in the dark. The bottles remained sealed throughout the incubation and gas headspace (100 μL) was periodically measured (every 2 days prior to day 8 followed by weekly measurements) for CO_2 and CH_4 concentrations for 4 weeks.