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RESEARCH ARTICLE

IMPACTS OF FIRE ON MICROBIAL CARBON CYCLING IN SUBTROPICAL WETLANDS

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ABSTRACT

Fire is a major determinant of the global carbon (C) balance. While it is known that C is lost through organic matter combustion, the effect fire has on soil C biogeochemistry is unclear. Studies investigating the role of fire on C greenhouse gas production (CO_2 and CH_4) have been conducted in forested and grassland ecosystems, yet research in wetlands has been limited. With their high potential for C storage, wetland ecosystems are important in C cycling while simultaneously serving as the largest single CH_4 source in the world. Wildfires typically consume a majority of the above-water biomass in wetland systems that result in direct C losses, but the subsequent implications for C processing are unknown. Thus, understanding C cycling in wetlands regularly maintained or influenced by fire is critical to meeting C sequestration management objectives. This study focused on a fire-adapted wetland ecosystem undergoing restoration from agricultural impacts within the Everglades National Park, Florida, USA. Within the site, the effects of prescribed fire on C cycling (organic C, extractable organic C, enzyme activity, CO_2, and CH_4 production) were monitored in a restored (high-phosphorus [P]) and reference (low-P) wetland at both high and low elevations. Because fire can affect both C and P forms and availability, the objective of this study was to investigate the short- (two-day) and long-term (one-year) effect of fire on C cycling in subtropical wetlands soils of varying soil nutrient concentrations. Initially (two days post fire), C cycling was stimulated in both soils. However, stimulation of CO_2 and CH_4 production was observed only at the reference (low-P) site. This result suggests that fire may have an adverse effect on C cycling in low-P soils, initially augmenting C greenhouse gas production. Minimal heat transfer coupled with constant microbial biomass suggests that nutrients may have been a regulating factor in this process. After one year, no fire effect was distinguishable on C parameters from reference sites, yet variable effects were observed in restored soils. This suggests that C cycling in reference sites may recover more quickly than restored sites. The ultimate consequences of fire on C cycling in these wetlands systems are dependent on time and are strongly influenced by pre-fire site conditions.

Keywords: ash, carbon, char, CO_2, Everglades, marl, methane, phosphorus, temperature

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INTRODUCTION

Fires (natural and prescribed) can drastically modify ecosystem function by affecting soil nutrients, microbial activity and community, and the global carbon (C) balance. The extent to which an ecosystem is modified (post fire) is controlled largely by fire intensity, severity, and type. Fire intensity (reaction intensity) is defined as the rate of energy release by the combustion of fuels over a given area (kW m^{-2}), whereas fire severity is a measure of above- and belowground biomass change. Fire type defines the strata of fuel through which typical fires burn. In the case of gramminoid- and shrub-dominated wetland ecosystems, fire commonly moves through the densely spaced, continuous fine surface fuels, consuming the majority of biomass above the surface level of the water. These three factors, along with post-fire climate, area burned, and additional disturbance, dictate the ecosystem response (Keeley 2009) and are crucial when evaluating and predicting fire effects.

With fire frequency hypothesized to increase with global climate change (Westerling *et al.* 2006, Krawchuck *et al.* 2009), understanding how this process may affect greenhouse gas production and the C balance is crucial. While most fire data have been compiled from forested (White 1986, Covington and Sackett 1992, DeLuca and Sala 2006, Allison *et al.* 2010) and grassland ecosystems (Knapp *et al.* 1998, Xu and Wan 2008, Toma *et al.* 2010), limited data from wetlands have been documented (Levine *et al.* 1990, Smith *et al.* 2001, Nakano *et al.* 2006). Wetlands are known to be the largest single source of CH_4 in the world (IPCC 2007); thus, slight changes in these ecosystems may have large-scale consequences to greenhouse gas accumulation.

Fire can affect biogenic CH_4 production by modifying microbial biomass (soil sterilization) and nutrient availability and forms. Soil nutrients can be modified through removal, volatilization, and deposition. With fire temperatures ranging from 50 °C to >1500 °C, heat release will vary greatly (reviewed by Neary *et al.* 1999) and can affect microbial and soil parameters differentially. During a fire, nutrients experience one of three fates, to: (1) remain as incompletely burned organic matter (as char), (2) be lost to the atmosphere, or (3) be redeposited as organic matter derived ash (Boerner 1982). White *et al.* (1973) found nitrogen (N) volatilization to occur at fire temperatures greater than 200 °C, although N loss has been reported at temperatures as low as 120 °C (Hart *et al.* 2005). This suggests that N volatilization may be influenced by site and vegetation characteristics. In contrast, soil phosphorus (P) responds differently to fire when compared to N because there is no appreciable volatilization reported. Thus, P integrity is preserved at high temperatures ($\geq$500 °C) (White *et al.* 1973), although the form may be altered (Smith *et al.* 2001). As a result, a direct fire response may result in alteration of nutrient concentrations and availability directly affecting microbial survivorship.

Increased understanding of the effect of fire on C loss processes is needed especially in fire-adapted wetland ecosystems that are maintained through prescribed burning and wildfire. The objective of this study was to investigate both the immediate (two days post fire, one month post fire) and longer (one year post fire) term effects of fire on soil C processing in two fire-adapted subtropical wetlands that vary in soil nutrient (P) concentrations and are maintained through regular prescribed burning. Addition of P following fire is likely due to ash and char deposition; thus, we hypothesized that ecosystems that vary in soil P concentrations would respond differently to fire. More specifically, a greater stimulation in CO_2 and

CH_4 greenhouse gas production in low- relative to high-P soils would be evident as microbial P limitation would be alleviated.

METHODS

Study Area

The effect of prescribed burning on soil biogeochemical processing was tested in the Hole-in-the-Donut (HID) region of Everglades National Park of southern Florida, USA (Figure 1). Soils in this region belong to the Perrine and Biscayne marl soil class and are calcareous subtropical wetlands with poor to very poor draining characteristics (USDA 1996). These wetlands are typically flooded 6 to 8 months of the year. This land was heavily farmed until 1975 when the farms were abandoned and the park purchased the land. In response to land use change, soil disturbance, and nutrient addition (fertilization), the land was overgrown by the aggressively invasive, root-sprouting small tree-shrub, *Schinus terebinthifolius* Raddi (Brazilian pepper). Through an extreme wetland restoration approach, the land was scraped to bedrock (complete removal of soil and vegetation). Scraping of the soils (and vegetation removal) began in 1989 and is still continued today with the overarching restoration goal to remove the invasive vegetation, *S. terebinthifolius*, and discourage regrowth (Ewel *et al.* 1982, Smith *et al.* 2011, Inglett and Inglett 2013).

In this study, soils restored by extreme restoration in 2000, and a native reference site

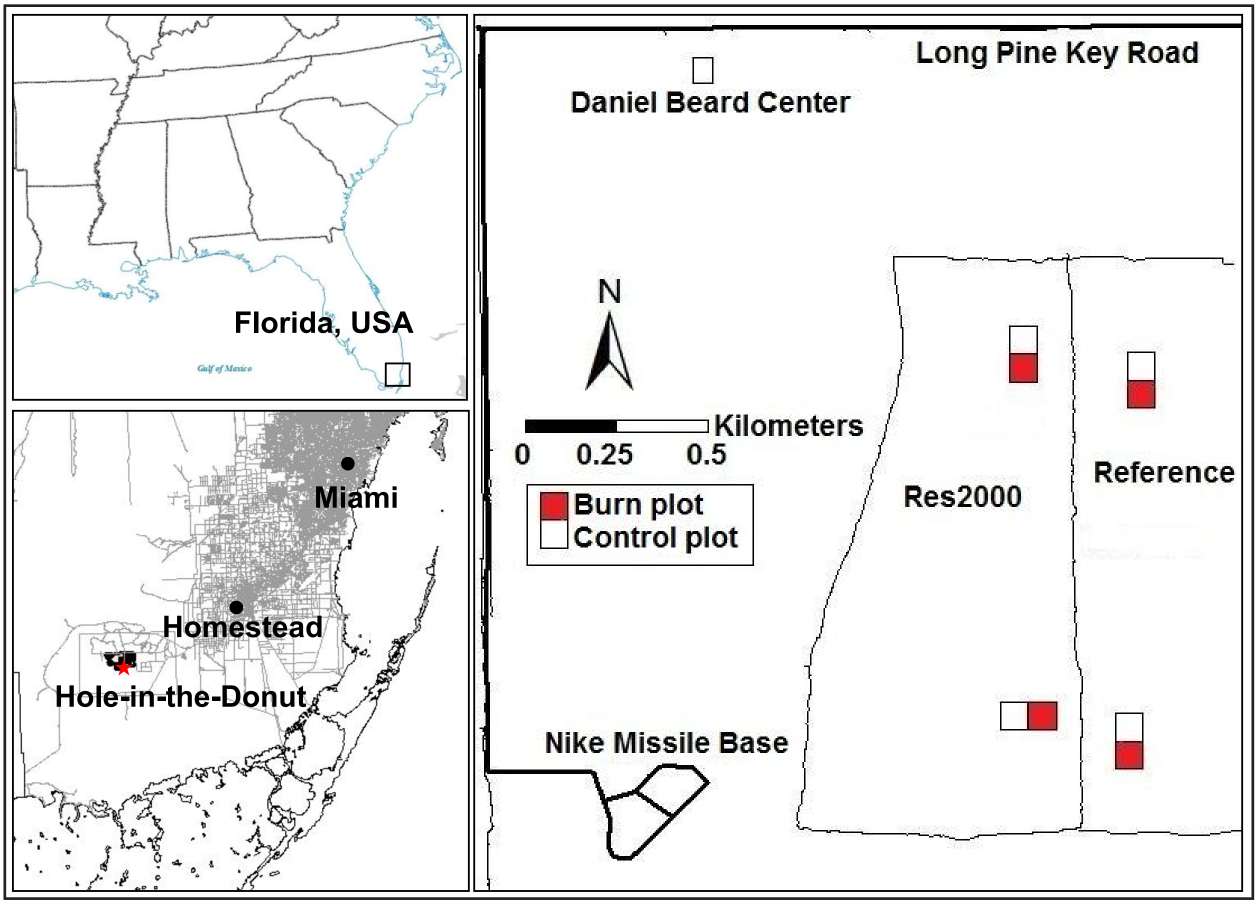

Figure 1. Map of the sampling locations and prescribed fire experimental design within the Hole-in-the-Donut region of Everglades National Park, Miami-Dade County, Florida, USA. Res2000 is the restored wetland. Map prepared by Liao *et al.* 2013.

(never farmed or fertilized) were chosen to investigate the effects of fire on C cycling. Two main differences between the restored and reference site are (1) the dominant vegetation, and (2) the soil P concentrations. Vegetation at the restored site consisted of shrubs and grasses including *Baccharis halimifolia* L. and *Andropogon* spp., whereas the reference site was dominated by sedges and grasses (*Cladium* spp., and *Muhlenbergia* spp., respectively) similar to oligotrophic wetlands within the greater Everglades (Smith *et al.* 2011). Soils at the restored site contained high P (~700 mg kg^{-1}) concentrations relative to the low soil P concentrations (150 mg kg^{-1}) in the reference soil (P. Inglett, University of Florida, unpublished data).

Fire Characteristics

The restored and reference sites were burned on 4 May 2010. Field sites received 1.27 mm of precipitation four days prior to the burn. All desired conditions were met including a dry bulb temperature ranging from 86 °C to 89 °C, relative humidity between 55 % to 75 %, a cloud cover of 30 % to 50 % with wind speed between 0.89 m s^{-1} and 5.36 m s^{-1}, and fine fuel moisture averaging 8 %. Wind direction was predominately S or SSE. The fire was completed through manual and aerial ignition and was ignited as a head fire with a rate of spread between 5 m min^{-1} to 11 m min^{-1}. Fire temperatures in the restored site reached 190 °C and 140 °C at high and low elevations at ground level, respectively. However, greater increases in temperature were recorded from reference wetlands, with temperatures reaching 425 °C and 190 °C at high and low elevations, respectively.

Data Collection

Two adjacent 30 m^2 plots were established at the restored site and reference wetland sites at both high and low elevations during the dry season. One 30 m^2 plot at each high and low elevation site was excluded from fire with a 2 m buffer strip that was cut on 8 to 10 April 2010 (Figure 1). At the time of plot preparation, soil samples were collected to determine baseline conditions from all designated burn and non-burn plots. To monitor the soil and surface level fire temperature, six thermocouple data loggers were placed on the soil surface and installed at a 1 cm soil depth, where the majority of microbes reside (Neary *et al*. 1999; P. Inglett, unpublished data).

Soil Sampling and Analysis

Soils were collected from 0 cm to 5 cm depths (or until bedrock) using a spatula, transferred to a bag and placed on ice, and transported to the University of Florida Wetland Biogeochemistry Laboratory for processing within 1 to 2 days of sampling. Soil samples were sieved through a 2 mm mesh to remove rocks and roots. Representative aliquots were removed from each sample ($n = 3$ per site) and analyzed for soil nutrients (carbon and phosphorus), microbial biomass carbon (MBC), ß-glucosidase enzyme activity (BGA) and cellobiohydrolase (CBH) enzyme activity, respiration potentials (aerobic and anaerobic CO_2), and methanogenesis. The prescribed fire occurred on 4 and 5 May 2010 and our post fire sampling began on 6 to 7 May (two days post fire). Additional samples were retrieved 11 June 2010 (one month post fire) and 25 May 2011 (one year post fire).

Soil organic C was estimated using loss on ignition (550 °C combustion for four hours) assuming a 45 % organic carbon (OC) of organic matter (OM) content factor (Wright *et al*. 2008). Microbial biomass carbon and nitrogen was analyzed by extraction following chloroform fumigation (Vance *et al*. 1987). Briefly, soil samples were extracted with 0.5 M K_2SO_4 after being incubated with (fumigated) and without (control) chloroform. Filtered (0.45 µm) extracts were analyzed for total extractable organic carbon (TOC) using a 5050A TOC auto-analyzer (Shimadzu Corp., Columbia, Maryland, USA; EPA method 415.1 [USEPA 2012]). The difference in extractable TOC between the fumigated and non-fumigated samples was considered to be the MBC following correction with an extraction efficiency (K_{EF}) of 0.37 (Sparling *et al*. 1990). Soil total phosphorus (TP) was determined by combusting soils at 550 °C, extracting ash with 6 M HCl (Anderson 1976), and analyzing extracts with a colorimetric method (EPA method 365.1 [USEPA 2012]) using a Technicon^{TM} Autoanalyzer (SEAL Analytical, Mequon, Wisconsin, USA).

Microbial respiration and methanogenesis. Aerobic soil respiration was measured by the base trap method (modified from Coleman 1973). Briefly, 2 g dry weight soil aliquots were sealed in 120 ml pyrex glass bottles containing 0.5 M NaOH base traps (modified from Coleman 1973). Traps were changed on days 1, 2, 3, and 4, and values for each time interval were summed to calculate cumulative respiration. Soil-free controls were used to account for background CO_2 levels and subtracted from soil respiration determinations. Upon removal, the base-trapped CO_2 was released by acidifying the solution (3 M HCl), and the released CO_2 was analyzed by gas chromatography.

To quantify anaerobic CO_2 and CH_4 production, 2 g dry weight soil and 10 ml of double deionized water (DDI) were combined in 30 ml conical tubes ($n = 3$ per site), sealed with butyl rubber stoppers and aluminum crimp tops, flushed with oxygen-free N_2 to ensure anaerobic conditions, and stored in the dark. After a 24 hour incubation period, gas headspace was measured, and periodically re-measured over the next 30 days, on a gas chromatograph to obtain a linear phase of gas production.

Gas analysis. Methane headspace was detected by a Shimadzu gas chromatograph-8a fitted with a flame ionization detector (160 °C; injection temperature 110 °C), N_2 as the carrier gas, and a 1.6 m (45/60 mesh) Carboxen-1000 column (Supelco Inc., Bellefonte, Pennsylvania, USA). A Shimadzu gas chromatograph-8a fitted with a thermal conductivity detector (column injection 120 °C; 40 °C oven temperature), He as the carrier gas, and a 1.83 m (80/100 mesh) Porapak-N column (Supelco Inc.) was utilized to analyze CO_2 headspace. Calibration curves were determined via standard gas mixtures (Scott Specialty Gases, Plumsteadville, Pennsylvania, USA) multiple times throughout each sampling event.

Enzyme Activity

Soil enzyme activity of cellobiohydrolase (CBH) and β-glucosidase (BGA) were measured fluorometrically with methylumbelliferone fluorophore on a BioTek® model FL 600 plate reader (BioTek Instruments, Inc., Winooski, Vermont, USA) following 100-fold soil dilutions with DDI, similar to Inglett *et al.* (2011). Briefly, a 500 μM substrate-fluorophore solution was added to the soil solution and incubated in the dark for two hours. Following the incubation, fluorescence was measured at an excitation of 350 nm and emission of 450 nm. Soil quenching curves were prepared to detect any possible quenching that may have occurred throughout the incubation. Methylumbelliferone standard curves were prepared and used to determine enzyme activity from the fluorescence. Activities were reported as nmoles MUF g^{-1} MBC h^{-1}.

Data Analysis

All statistical analyses were performed in JMP v. 8.0 (SAS Institute, Cary, North Carolina, USA). Initial differences in soil nutrient concentrations and microbial parameters between pre-fire burned plots and control plots were determined via one-way analysis of variance (ANOVA). Similarly, the fire effect on soil nutrients and microbial parameters were investigated via one-way ANOVA by comparing values from sites that were burned with values from an adjacent unburned control sites. In addition, differences in parameters within sites at different elevations were compared via one-way ANOVA. Differences between means ($\alpha < 0.05$) were assessed via Tukey-Kramer means comparison. Parameters were compared at each sampling time; thus, each sampling event was analyzed independently.

RESULTS

Soil Parameters

Soil organic carbon. Organic carbon was elevated in restored soils relative to reference soils (Table 1). In the restored sites (high and low elevation), fire had no effect on soil OC content when compared with the large fluctuations evidenced in the control soils. This trend was also observed in the reference high-elevation site. While no difference in OC was evident between burned soils and control soils at the reference low-elevation site in the short term (≤1 month), a slight elevation was detected ($P < 0.05$; Table 1) at one year.

Extractable organic carbon. In the high-elevation restored site, fire increased the extractable TOC from (350 ± 17) mg TOC kg^{-1} soil (control plot) to (451 ± 25) mg TOC kg^{-1} soil ($P < 0.05$). Although there was no fire effect on TOC one month post fire, an additional 64 mg TOC kg^{-1} soil was quantified in burned sites after one year ($P < 0.05$; Table 1). In contrast, there was no immediate fire effect (≤1 month) on extractable TOC at the lower elevation restored site, although concentrations decreased by 82 mg TOC kg^{-1} soil in burned plots relative to control plots one year following the fire ($P < 0.05$; Table 1).

Similar to the high-elevation restored site, fire increased TOC from (298 ± 20) mg TOC kg^{-1} soil to (381 ± 8) mg TOC kg^{-1} soil in the high elevation reference site two days following the fire ($P < 0.05$), although this response was eliminated after two days. At the low-ele-

Table 1. The effect of fire on soil organic carbon (OC) and extractable total organic carbon (TOC) (0.5 M K_2SO_4) from restored and reference wetlands within the Hole-in-the-Donut, Everglades National Park, Florida, USA. Samples were collected two days (6 and 7 May 2010), 1 month (11 June 2010), and 1 year (25 May 2011) following the prescribed burn. (Average ± SE, $n = 3$, * = $P < 0.05$).

		Soil OC (g kg^{-1})		Extractable TOC (mg kg^{-1})	
Site	**Time after fire**	**Burn**	**Unburned**	**Burn**	**Unburned**
Restored-high elevation	2 days	74 (5)	66 (6)	451 (26)*	350 (17)
	1 mo	82 (5)	133 (32)	350 (32)	398 (23)
	1 yr	91 (3)	90 (3)	478 (11)*	414 (22)
Restored-low elevation	2 days	77 (5)	67 (5)	336 (12)	336 (21)
	1 mo	105 (21)	103 (14)	340 (16)	372 (9)
	1 yr	102 (10)	86 (6)	377 (24)	463 (16)*
Reference-high elevation	2 days	60 (9)	66 (11)	381 (8)*	298 (20)
	1 mo	49 (4)	59 (3)	322 (12)	333 (15)
	1 yr	54 (1)	59 (5)	348 (32)	332 (8)
Reference-low elevation	2 days	58 (6)	50 (4)	279 (18)	239 (17)
	1 mo	62 (3)	66 (3)	305 (20)	301 (10)
	1 yr	58 (1)*	51 (1)	305 (15)	272 (22)

vation reference site, fire had no significant effect on extractable TOC over the one year period (Table 1).

Enzyme Activity

Cellobiohydrolase enzyme activity. Cellobiohydrolase activity was not affected by fire in the restored high-elevation site immediately (two days) following fire; rates averaged (13 ± 2) nmoles MUF g^{-1} MBC h^{-1} and (12 ± 1) nmoles MUF g^{-1} MBC h^{-1} from burned and control plots, respectively. At one month, CBH activity was suppressed by 28 % ± 4 % in the burned plot relative to the control plot ($P < 0.05$; Figure 2A), although no remaining difference was observed at one year. At low elevation restored sites, CBH was not affected by fire (Figure 2C). Similarly, fire had no significant effect on CBH at reference sites, although the burn:control ratio suggests increasing activity post fire (Figures 3A, 3C).

βeta-glucosidase enzyme activity. In the high elevation restored site, activity of β-glucosidase increased from (75 ± 12) nmoles MUF g^{-1} MBC h^{-1} (control soils) to (100 ± 5) nmoles MUF g^{-1} MBC h^{-1} (burned soils) ($P < 0.05$). The burn:control ratio, although not significant over time, suggests decreasing activity in the burned plot relative to the control plot (Figure 2B). At the low-elevation site, a similar decreasing burn:control ratio was observed, although no significant difference between burn plot and control plot activities were evidenced at any time point (Figure 2D). No significant effect of fire on BGA was detected at the reference site, although trends in the burn:control ratio suggest decreasing activity in burned plots with time post fire (Figures 3B, 3D).

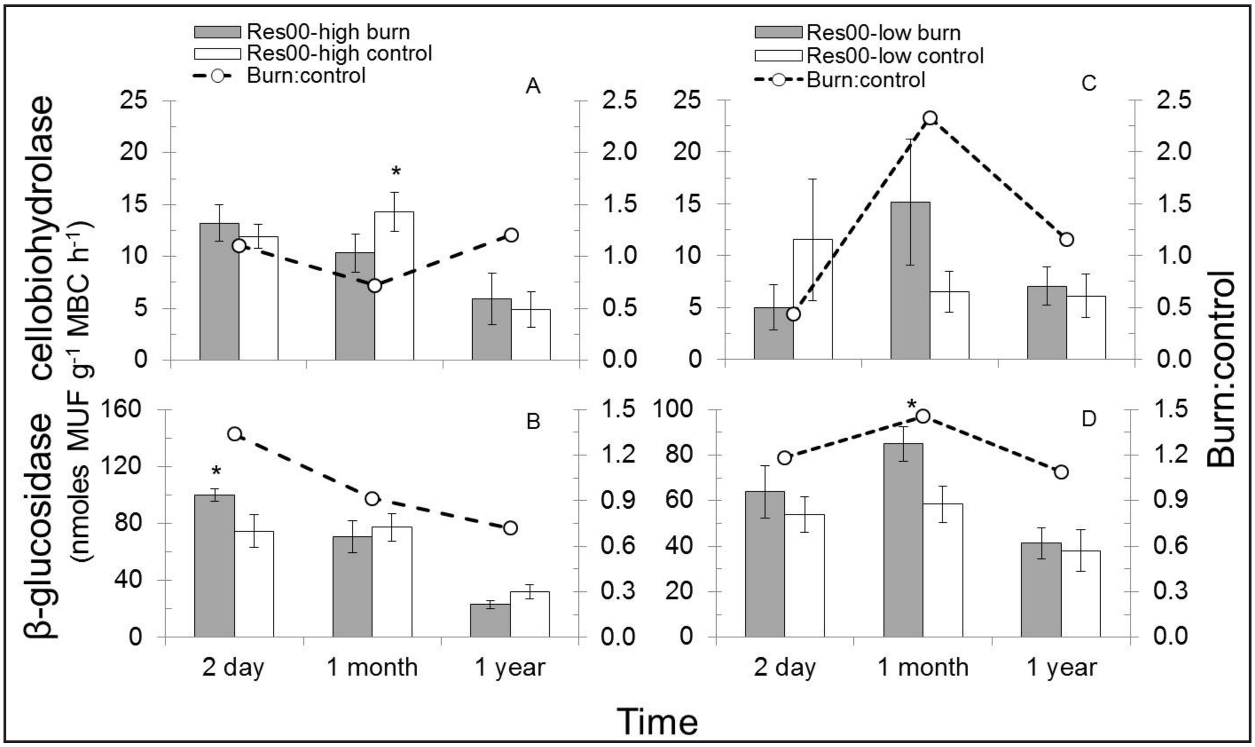

Figure 2. Effect of fire on C enzyme activity (cellobiohydrolase and β-glucosidase) two days (6 and 7 May 2010), one month (11 June 2010), and one year (25 May 2011) post fire in the restored high- and low-elevation sites within the Hole-in-the-Donut region of Everglades National Park, Miami-Dade County, Florida, USA. (Average ± SE, $n = 3$, * = P <0.05).

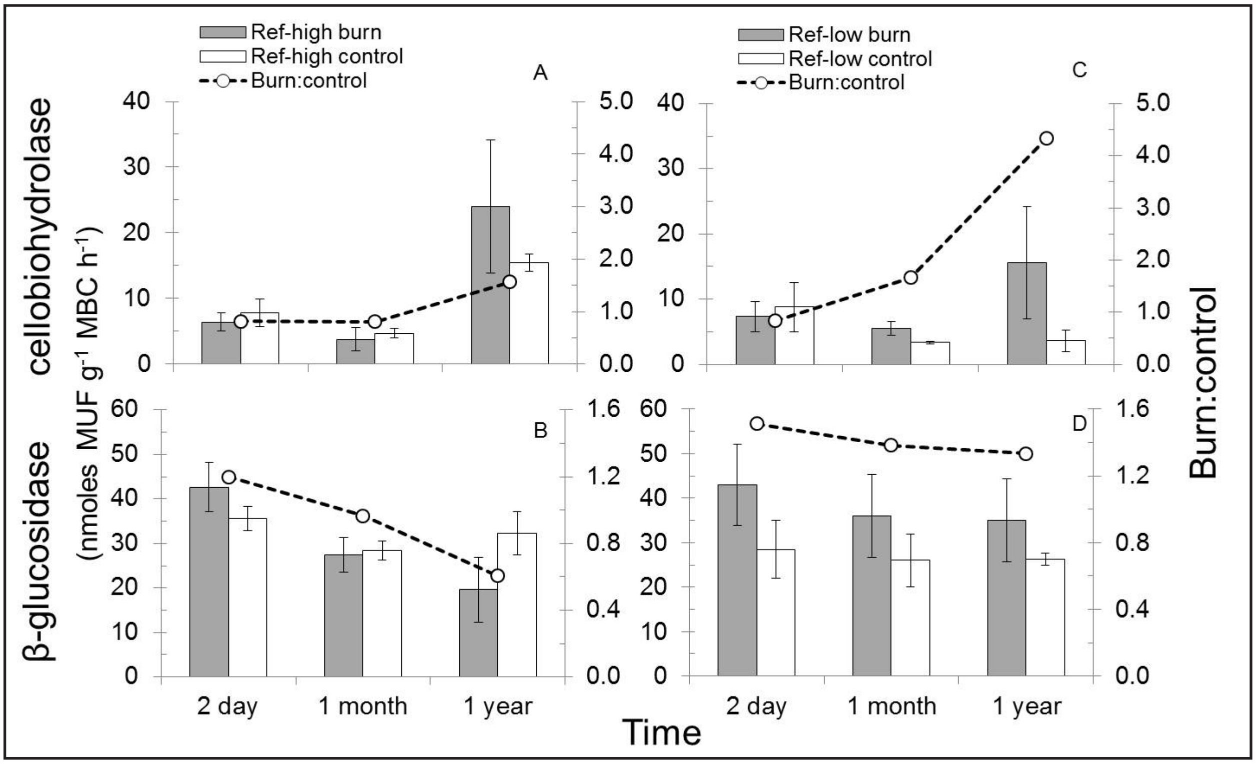

Figure 3. Effect of fire on C enzyme activity (cellobiohydrolase and β-glucosidase) two days (6 and 7 May 2010), one month (11 June 2010), and one year (25 May 2011) post fire in the reference high- and low-elevation sites within the Hole-in-the-Donut region of Everglades National Park, Miami-Dade County, Florida, USA. (Average ± SE, $n = 3$).

Microbial Respiration

Aerobic CO_2 production. Fire stimulated aerobic CO_2 production in the high elevation restored site two days post fire, with rates averaging (565 ± 126) mg CO_2-C kg^{-1} MBC d^{-1} and (390 ± 5) mg CO_2-C kg^{-1} MBC d^{-1} from burn and control soils, respectively ($P < 0.1$). At one month, a 45 % ± 25 % decrease in CO_2 production from burned soils relative to control soils was observed ($P < 0.05$); however, no difference was detected at one year (Figure 4A). In the restored low-elevation site, there was an increase in CO_2 production from burned plots at two days ($P < 0.05$); however, data taken prior to the fire detected elevated production from the burned plot, suggesting that this increase at two days is likely misleading. In addition, there was no effect of fire on this process at one month or one year following the fire (Figure 4D). At reference sites, fire had no significant effect on CO_2 production (Figures 5A, 5D).

Anaerobic CO_2 production. Two days following the fire there was no effect on anaerobic CO_2 production from the high elevation restored site. At one month, CO_2 was suppressed (64 % ± 12 %) in burned plots ($P < 0.05$), a result that was also observed under aerobic conditions. At one year, fire had no detectable effect on this parameter (Figure 4B). At low elevations, fire had no significant effect on CO_2 production at two days. Similar to results at the high elevation, CO_2 was suppressed (28 % ± 9 %) in the burned plot at one month ($P < 0.05$). At one year, this trend persisted ($P < 0.05$); a decreasing burn:control ratio suggests CO_2 production decreased with time following the fire (Figure 4E).

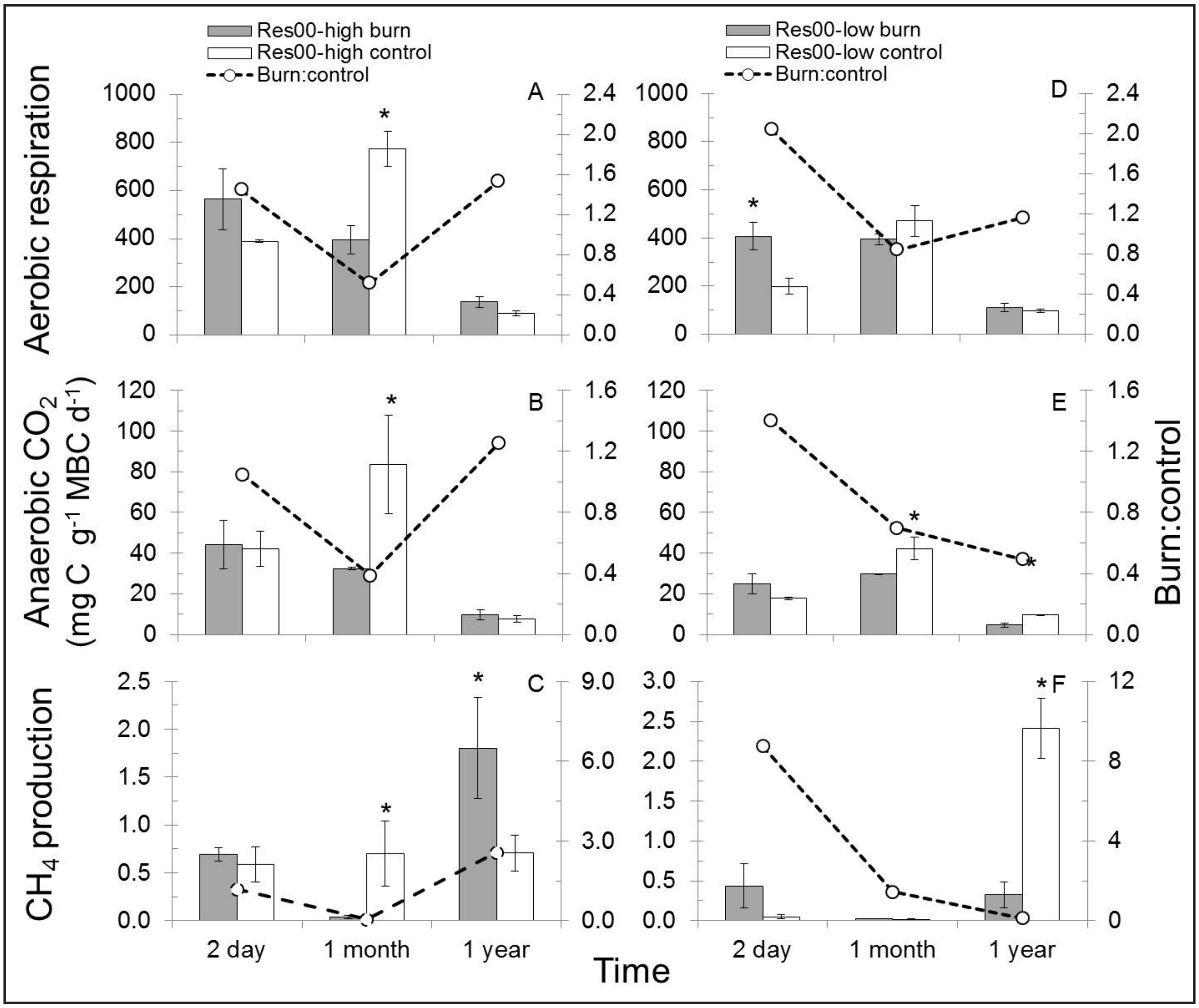

Figure 4. Effect of fire on aerobic (CO_2) and anaerobic (CO_2, CH_4) respiration potentials two days (6 and 7 May 2010), one month (11 June 2010), and one year (25 May 2011) post fire in the restored high- and low-elevation sites within the Hole-in-the-Donut region of Everglades National Park, Miami-Dade County, Florida, USA. (Average ± SE, $n = 3$, $*P < 0.05$).

At the high elevation reference site, fire increased CO_2 production by 89% ± 19% ($P < 0.05$). Similar to trends in the restored site, CO_2 production was suppressed in burn plots by one month. Rates averaged (16 ± 1) mg CO_2-C g^{-1} MBC d^{-1} and (53 ± 14) mg CO_2-C g^{-1} MBC d^{-1} from burned soils and control soils, respectively ($P < 0.05$); however, by one year, no effect of fire on this parameter was observed (Figure 5B). At the low-elevation site, CO_2 was elevated following fire; rates increased from (27 ± 8) mg CO_2-C g^{-1} MBC d^{-1} to (39 ± 4) mg CO_2-C g^{-1} MBC d^{-1} in control plots and burned plots, respectively ($P < 0.1$). At one month, CO_2 was still elevated in the burned plot ($P < 0.05$), although this trend was no longer observed one year following the fire. The burn:control ratio suggests that CO_2 from the burned plot was steadily increasing relative to production from the control plot with time (Figure 5E).

Methane production. Methane production was not affected by fire in the high elevation restored site two days following the fire. Similar to the suppression in aerobic and anaerobic

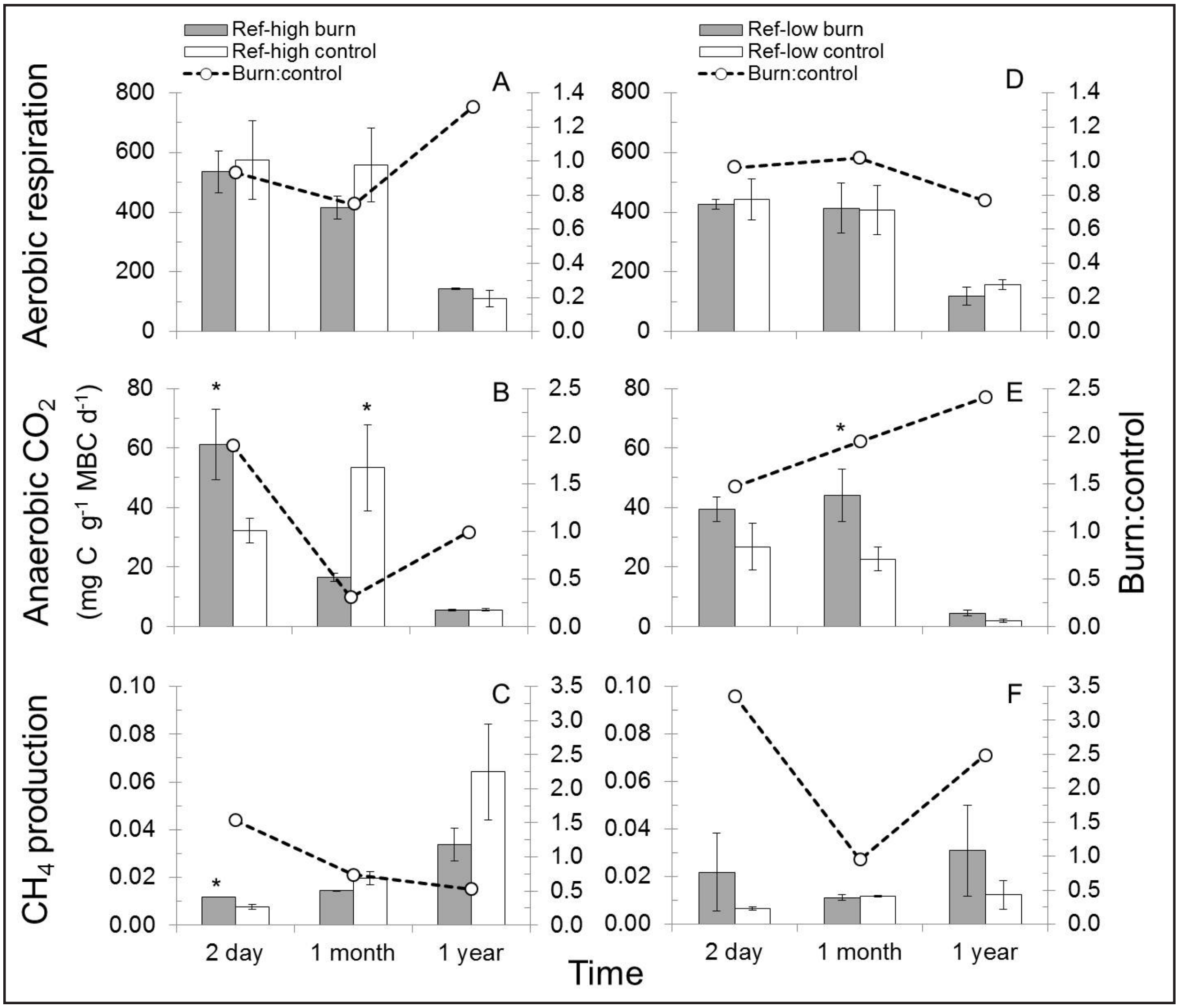

Figure 5. Effect of fire on aerobic (CO_2) and anaerobic (CO_2, CH_4) respiration potentials two days (6 and 7 May 2010), one month (11 June 2010), and one year (25 May 2011) post fire in the reference high- and low-elevation sites within the Hole-in-the-Donut region of Everglades National Park, Miami-Dade County, Florida, USA. (Average ± SE, $n = 3$, $*P < 0.05$).

CO_2 production at one month, CH_4 was also suppressed in burned plots ($P < 0.05$), with rates averaging 0.36 mg CO_2-C g^{-1} MBC d^{-1} and 0.7 mg CO_2-C g^{-1} MBC d^{-1} (67% ± 28%) in burned plots and control plots, respectively. In contrast, at one year, production of CH_4 was stimulated in burned soils, increasing from (0.7 ± 0.2) mg CH_4-C g^{-1} MBC d^{-1} to (1.8 ± 0.5) mg CH_4-C g^{-1} MBC d^{-1} in control plots and burned plots, respectively ($P < 0.05$; Figure 4C). Trends in the burn:control ratio from the low-elevation site suggest a decrease in production from burned plots relative to production from control plots with time. At two days and at one month, no significant burn effect was detected in CH_4 production. Similar to the observed trend in anaerobic CO_2 production at one year, CH_4 was also suppressed in burned plots ($P < 0.05$), resulting in a decreased production rate from (2.4 ± 0.4) mg CH_4-C g^{-1} MBC d^{-1} to (0.3 ± 0.2) mg CH_4-C g^{-1} MBC d^{-1} from burned plots and control plots, respectively (Figure 4F).

In the high elevation reference site, CH_4 production was initially (two days) stimulated (57% ± 22%) by fire ($P < 0.05$); however, a decreasing trend in the burn:control ratio suggests that this stimulation was transitory, decreasing quickly with time (Figure 5C). At the low-elevation site, no effect of fire on CH_4 production was detected at two days, one month, or one year (Figure 5F).

DISCUSSION

Immediate Response of Microbial and Soil Parameters to Fire

Close to 77% and 94% of the aboveground vegetation and litter was removed from the restored and reference wetlands, respectively (P. Inglett, unpublished data). This suggests a more complete burn, indicating higher fire severity, in the reference site relative to the restored site. Variation in the fire intensity can affect microbial activities. In most grassland fires, temperatures can approach 300 °C on the soil surface; however, at 1 cm depths, where most microbes reside (Neary *et al*. 1999), temperatures spike to ~60 °C, gradually decreasing with time (Ryan 2002), suggesting that microbial mortality in low-intensity grassland fires is minimal (Raison 1979, Neary *et al*. 1999, Hart *et al.* 2005). In mineral soils, Raison *et al.* (1985) observed less than a 15% transfer of aboveground heat to belowground heat. Nevertheless, in response to fire, most soils increase in temperature, although the amplitude of this temperature change can vary immensely.

Increasing soil temperatures in response to fire can result in a reduction of the soil moisture content (Tix *et al.* 2006), further inhibiting survival of some microbial communities. This change in temperature and moisture content may provide a competitive advantage to microbes that flourish or can withstand high temperature and desiccation. There was a reduction in the soil moisture content from the burned plot relative to the control restored plot at the high elevation site ($P < 0.05$; data not shown), although there were no other differences in soil moisture content detected. As the soil begins to warm, the active sites of microbial-derived enzymes begin to lose function prior to the protein denaturing (Wallenstein *et al.* 2011), suggesting that short-term temperature variation may reduce active-site function, although temperatures (in this case) may not be extreme enough to denature the protein. An elevated temperature post fire may persist for months or even years (Neary *et al*. 1999), mostly due to increased heat absorption from minimal vegetation shading (Rivard and Woodward 1989).

In our study sites, temperature transfer and the effect on microorganisms would likely be more extreme in the restored site due to shallower soil depths relative to the deeper reference-site soils. For example, in the restored site, soil depths averaged 1.7 cm. If a lethal temperature penetration of 1 cm is assumed, then 60% of the soil collected from the restored site was likely exposed to high temperatures, while only 20% of soil collected from reference wetlands (0 cm to 5 cm) would experience similar high temperatures. Temperatures within the HID soils fluctuated largely at 1 cm depths, extending from 20 °C to 40 °C in both sites across spring and summer months (data not shown). Furthermore, soil temperatures did not exceed 70 °C at 1 cm depths (P. Inglett, unpublished data) during the fire event, suggesting that microbial activity would be minimally affected (Ryan 2002).

Although aboveground environmental conditions were uniform while burning (air temperature, humidity, etc.), variation in site vegetation and production of vegetation derived residues of ash and char may explain the post-fire differences in microbial and soil C parameters. Wind-driven surface fires, especially those burning through grass-dominated fuelbeds, are expected to have a minimal effect on soil OM (DeBano and Klopatek 1988), as observed in restored and reference wetlands, likely due to a fast rate of spread and a short fire residence time. In addition, low soil OM at these sites (10% to 20%) may discourage high severity burns, thus decreasing heat transfer to the soil.

The highest proportion of microbial activity is typically found within the top centimeter of soil (Neary *et al*. 1999). At depths greater than 2 cm to 3 cm, temperatures during and after fire can be similar to ambient temperatures (González-Pérez *et al*. 2004). For this reason, temperature data are important in determining the effect of fire on microbial biomass and associated parameters. It is common to observe no stimulation in soil MBC post fire in prairie (Ajwa *et al.* 1999) and forested ecosystems (Prieto-Fernández *et al.* 1998). We found no effect of fire on MBC in restored or reference sites following the fire, possibly due to the low fire intensity and minimal heat transfer to the soil (data not shown).

Following a fire, ecosystems with no change in MBC coupled with complete vegetation removal (substrate reduction) would suggest that C enzyme activity would be minimally affected. However, activity of C enzymes are considered to be a main regulator of decomposition (Sinsabaugh *et al.* 1993), which is why we quantified the activities of two C enzymes in the current study. One of these enzymes (CBH) targets the degradation of more complex OM, whereas the other C enzyme (BGA) can only degrade more decomposed OM. Following fire, many studies have quantified BGA activity with varying results (Ajwa *et al.* 1999, Boerner *et al.* 2003, Gutknecht *et al.* 2010); however, limited data on CBH has been reported (Gutknecht *et al.* 2010). Immediately following the fire, there was no response in CBH from the restored site. It is plausible that a reduction in vegetation-derived complex C inputs reduced the substrate availability necessary to stimulate CBH production. The products of CBH activity provide the substrates necessary to fuel BGA activity. For this reason, the production of BGA is typically related to CBH activity. Because CBH (g^{-1} MBC) was not stimulated post fire, an increase in substrates fueling BGA was not expected. However, at the high elevation, stimulation of BGA was evident ($P < 0.05$; Figure 2B). Elevated BGA activity suggests that an increased proportion of degraded OM compounds (substrates) were present at the burned plot relative to the control plot. An increase in decomposed OM may be a response to the addition of incompletely combusted OM and litter material post fire. It may have also originated from relocation of C within the vegetation during the fire, which may have increased the proportion exuded from the roots.

Similar to results from the restored site, CBH (g^{-1} MBC) was not affected by fire in reference wetlands, likely due to decreased quantity and complexity of the remaining OM. When taking into account the higher pre-burn activity from control plots, the stimulation of BGA was likely masked, although changes in BGA are not commonly reported post fire (Boerner *et al.* 2005, Gutknecht *et al.* 2010). This suggests a tight coupling between C enzyme activities in the reference site.

Elevated extractable TOC may be observed post fire in response to an increase in BGA, degradation of microbial biomass, or as a leachate from incompletely combusted OM. Anderson *et al.* (2004) observed elevated TOC from burned soils relative to control soils for 90 days following a fire. In the current study, there was stimulation of extractable TOC at high-elevation wetlands regardless of site ($P < 0.05$; Table 1). Increased TOC immediately following a fire may stimulate microbial decomposition processes (Anderson *et al.* 2 004).

Aerobic decomposition (CO_2) was stimulated in the restored burned plots regardless of elevation. However, the stimulation at the low-elevation site may be misleading because higher production originated from the burned plot prior to initiation of the experiment. Furthermore, the stimulation pre- and two days post fire was equivalent (~50%). The increase in BGA, extractable OC, and aerobic CO_2 production immediately following the fire at the high-elevation site suggests that an increased pulse of extractable OC was delivered to the burned plots, which may have been used to fuel aerobic processing. Furthermore, at the high-elevation site, there were positive correlations between TOC and BGA ($P < 0.01$). It has been hypothesized that the attack of BGA on OM would likely release TOC as a decomposition product (Freeman *et al.* 1997). A lack of stimulation in aerobic respiration from the reference sites coupled with enhanced TOC concentrations suggests that C substrate availability was not the main regulator of this process in reference wetlands.

A lack of stimulation in anaerobic CO_2 production was observed at the restored burned site, a result that has been reported previously on net soil CO_2 flux (Toma *et al.* 2010). We are unaware of any study that has quantified the effect of fire on anaerobic CO_2 production. Toma *et al.* (2010) concluded that there was no effect of burning on CH_4 production in a grassland ecosystem. This is in agreement with results from the restored site, suggesting that fire initially had a greater effect on aerobic processing as opposed to anaerobic processing in this site. In reference wetlands, CH_4 production was stimulated by the fire at the high-elevation site ($P < 0.05$), similar to Nakano *et al.* (2006). No difference in CH_4 production was detected between burn plots and control plots at the lower elevation. Levine *et al.* (1990) found CH_4 production to decrease immediately following fire, although a delayed stimulation (days 3 to 9 post fire) was observed, illustrating the value of repeated post-fire soil collection and analysis. We sampled the soil two days following the fire, thus our results in the reference site were similar to those of Levine *et al.* (1990). A change in C quality or availability may be fueling differences in anaerobic decomposition (Ajwa *et al.* 1999, Boerner *et al.* 2000). However, it is plausible that a change in soil P availability may have stimulated anaerobic decomposition in our low-P reference site. Although an initial increase in available P was not observed at the high elevation reference site, a significant decrease in the MBC:MBP ratio suggests a decrease in microbial P limitation ($P < 0.05$) initially post fire. In addition, soil total P was elevated in burned reference plots two days post fire.

Monitoring from One Month to One Year Post Fire

We concluded that stimulation of C parameters in response to fire were the greatest two days post fire regardless of site. Monitoring soil and microbial C parameters for a year following the fire provided a unique opportunity to conclude that each wetland responded differently to the fire. For example, at high elevations, C parameters quantifying decomposition (CO_2, CH_4) were suppressed after a different length of time depending on the site. In restored wetlands, decreased CO_2 and CH_4 production was observed at one month. At one year, the response of C parameters to fire varied greatly between sites and elevations. The results at one year illustrate the necessity of intensive and longer-term sampling. Unfortunately, due to difficulty in accessing the site and the long duration for laboratory incubations, more intensive sampling was not feasible in the current study.

Ecosystem recovery (post fire) is largely dependent on vegetation regrowth (species and time) (Hart *et al.* 2005). In our study sites, which were dominated by grasses, vegetation regrowth was quick relative to ecosystems dominated by woody vegetation such as forests. Two days post fire, grasses were already visible in the reference site. Quick regrowth of vegetation will negate many fire-driven responses such as elevated soil temperature, decreased moisture content, and reduced C substrate availability. This study suggests that nutrients derived from ash and char residues may have been more important in explaining differences in C parameters than heat transfer largely because MBC was not stimulated in burned plots (relative to the control) and heightened soil temperature did not persist.

Many studies have investigated the response of soil and vegetation C parameters to fire in multiple ecosystems (Nakano *et al.* 2006, Allison *et al.* 2010, Toma *et al.* 2010). Understanding the mechanisms that govern observed differences is difficult because fire characteristics and long-term data are rarely collected. As seen in the current study, most of the fire effect on microbial C processing was transient (within two days), suggesting that the timing of sampling is crucial. In grassland ecosystems, similar to the HID condition prior to burning, fire is capable of oxidizing a large proportion of OM. Overall, oxidation of aboveground OM was more complete in reference sites relative to restored sites (P. Inglett, unpublished data). Biomass removal was also greater from high elevation plots relative to low elevation plots. Thus, these differences may have altered the proportion of ash and char produced at each site and associated elevation, which may have explained the initial stimulation of C processing.

The response of soil and microbial C parameters to fire varied at each site. A minimal transfer of aboveground heat to the soil and no change in microbial biomass suggests that differences in C cycling post fire may have been in response to initial site differences and a fire-associated nutrient pulse. Our results suggest that C cycling in the HID was stimulated immediately following fire; however, stimulation of microbial processes (CO_2 and CH_4 production) were accentuated in reference (low-P) soils. Regardless of site, no loss in soil OC was detected; however, close to 100% of the aboveground biomass (vegetation and litter) was removed. After one year, the effect of fire was not detected on C parameters in the reference soils, suggesting that these processes recovered quickly following fire. In contrast, differences in C parameters (CH_4 and CO_2 production) from burned and control plots were still present one year post fire in restored soils. This suggests that the microbial communities may respond differently in this disturbed, high-P wetland. Differences may have been in response to woody vegetation present in the restored site, which may have altered ash and char production and subsequent nutrient addition. Our results highlight the different effects that fire can have on C cycling across short- and longer-term time scales. Increased C greenhouse gas production immediately following fire indicates that this process may enhance CO_2 and CH_4 production in the short term, suggesting that fire may have an adverse effect on C greenhouse gas production in low-P soils. Future studies are necessary to investigate if ash and char addition (nutrients) can explain the differences in soil C cycling observed in this study. A fire-derived nutrient pulse may explain differences in short- and long-term patterns of microbial C cycling, as well as differences between low- and high-P sites.

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