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**INFLUENCE OF HERBIVORE DAMAGE ON METHANE
EMISSION FROM EMERGENT AQUATIC MACROPHYTES**

ANTONELLA PETRUZZELLA

A dissertation submitted to the Institute of Biology through the Department of Ecology in partial fulfillment of the requirements for the degree of Master in Ecology at the Federal University of Rio de Janeiro.

Supervisor: Dr. Francisco de Assis Esteves

RIO DE JANEIRO, RJ, BRAZIL

MARCH, 2015

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"O
Homem é do
tamanho
do seu
sonho!"

Fernando Pessoa

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ABSTRACT

Among global ecosystems, wetlands are the largest natural source of atmospheric methane (CH₄). The major CH₄-emitting sites in wetlands are the littoral zones where emergent aquatic macrophytes dominate and participate in all three fundamental processes of CH₄ dynamics: production, oxidation and, particularly, emission. These processes vary greatly among plant species and life forms, and their net impact upon CH₄ emissions can range from negative to positive. Although the effects of herbivores on emergent aquatic macrophytes have been well documented, their effect on plant-mediated CH₄ emission, especially by insects, remains unknown. We performed a mesocosm experiment in which we simulated the damage caused by herbivorous insects and manipulated the density of damaged culms of *Eleocharis equisetoides* (4 levels – 0, 20, 50 and 100%) measuring the corresponding CH₄ flux, concentration and potential CH₄ production in the sediment. We hypothesized that an increased percentage of plants with simulated herbivory would be associated with increased CH₄ flux rates. Simulated herbivory positively affected CH₄ emissions, but only under high herbivory pressure. The average CH₄ flux from 100% damaged plants mesocosms was 3.5 higher than mesocosms with all intact plants (0% damage). These results indicate that physical damage on emergent macrophytes affects gas transport within the plants. A field survey in our studied system revealed that plant biomass consumed by herbivores is relatively low compared to the actual number of damaged plant culms. This particular result sheds light of the fact that herbivorous insects may have a disproportional effect on CH₄ emissions, due to physical damage, compared to their effect on plant biomass loss. In summary, our findings bring a new perspective to the influence of herbivory on the CH₄ and carbon cycling, especially regarding the role that herbivorous insects might play.

Key words: Herbivory, coastal lagoons, carbon cycle, gas transport, methanogenesis

RESUMO

Entre os ecossistemas globais, as áreas alagadas são a maior fonte natural de metano (CH_4) atmosférico. Os principais sítios emissores de CH_4 nas áreas alagadas são as zonas litorâneas, onde as macrófitas aquáticas emergentes dominam e participam dos três processos fundamentais da dinâmica do CH_4 : produção, oxidação e, especialmente, emissão. Estes processos variam muito entre espécies de plantas e formas de vida, e seu impacto sobre as emissões de CH_4 podem variar de negativo a positivo. Embora os efeitos dos herbívoros sobre as macrófitas aquáticas emergentes tem sido bem documentados, seu efeito nos fluxos de CH_4 mediados por planta, especialmente dos insetos, permanece desconhecido. Nós realizamos um experimento em mesocosmos em que nós simulamos o dano causado por insetos herbívoros e manipulamos a densidade de colmos danificados de *Eleocharis equisetoides* (4 níveis – 0, 20, 50 and 100%), medindo o correspondente fluxo de CH_4 , a concentração e produção de CH_4 potencial no sedimento. Nós hipotetizamos que um aumento da porcentagem de plantas com herbivoria simulada estaria associada com o aumento das taxas de fluxo de CH_4 . A herbivoria simulada afetou positivamente as emissões de CH_4 , mas somente sob altas pressões de herbivoria. O fluxo médio de CH_4 dos mesocosmos com 100% de plantas danificadas foi 3,5 vezes maior do que os dos mesocosmos com todas as plantas intactas (0% de dano). Estes resultados indicam que o dano físico causado nas macrófitas emergentes afeta o transporte de gases dentro das plantas. Um levantamento de campo no sistema estudado revelou que a biomassa de planta consumida pelos herbívoros é relativamente baixa se comparada com o número verdadeiro de colmos danificados. Esse resultado específico lança luz ao fato que insetos herbívoros podem ter um efeito desproporcional nas emissões de CH_4 , devido ao dano físico, comparado com seu efeito na perda de biomassa. Em síntese, nossos resultados trazem uma nova perspectiva para a influência da herbivoria sobre a ciclagem do carbono e do CH_4 , especialmente em relação ao papel que os insetos herbívoros devem exercer.

Palavras-chave: Herbivoria, lagoas costeiras, ciclo do carbono, transporte de gases, metanogênese

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GENERAL INTRODUCTION

1. Global Methane Cycle

1.1. Atmospheric Methane Sources

Methane (CH_4) is the second most important greenhouse gas present in the atmosphere and is 28 times more efficient at absorbing infrared radiation than carbon dioxide (CO_2) over a 100-year time frame (Foster et al. 2007; Ciais et al. 2013). Concentrations of CH_4 has nearly tripled since the beginning of the Industrial Era (defined as beginning in the year 1750), mainly due to growing of human activities, particularly in the areas of agriculture (e.g. rice cultivation), fossil fuel extraction and use, livestock and waste disposal (Wuebbles and Hayhoe, 2002; Ciais et al. 2013). Thus, small changes in its atmospheric concentration have large implications for future climate and have stimulated studies from terrestrial and aquatic environments (Butenhoff and Khalil, 2007; Bridgham et al. 2013; Carmichael et al. 2014).

CH_4 is released into the atmosphere by a wide variety of sources both natural and/or anthropogenic in origin (Figure 1). The total global CH_4 emission is estimated to be 542-852 $\text{TgCH}_4 \text{ yr}^{-1}$ (Ciais et al. 2013). Anthropogenic emissions arise from rice paddies agriculture, ruminant animals, sewage and waste, landfills, and fossil fuel use (coal mining, gas and oil industries) and their contribution are estimated to range between 50% and 65% of the total global emissions (Ciais et al. 2013). Major contributions of this emission rates come from rice, animals and waste with a total estimate of between 187 and 224 $\text{TgCH}_4 \text{ yr}^{-1}$ (Ciais et al. 2013) overtaking fossil fuel related emissions (Figure 1).

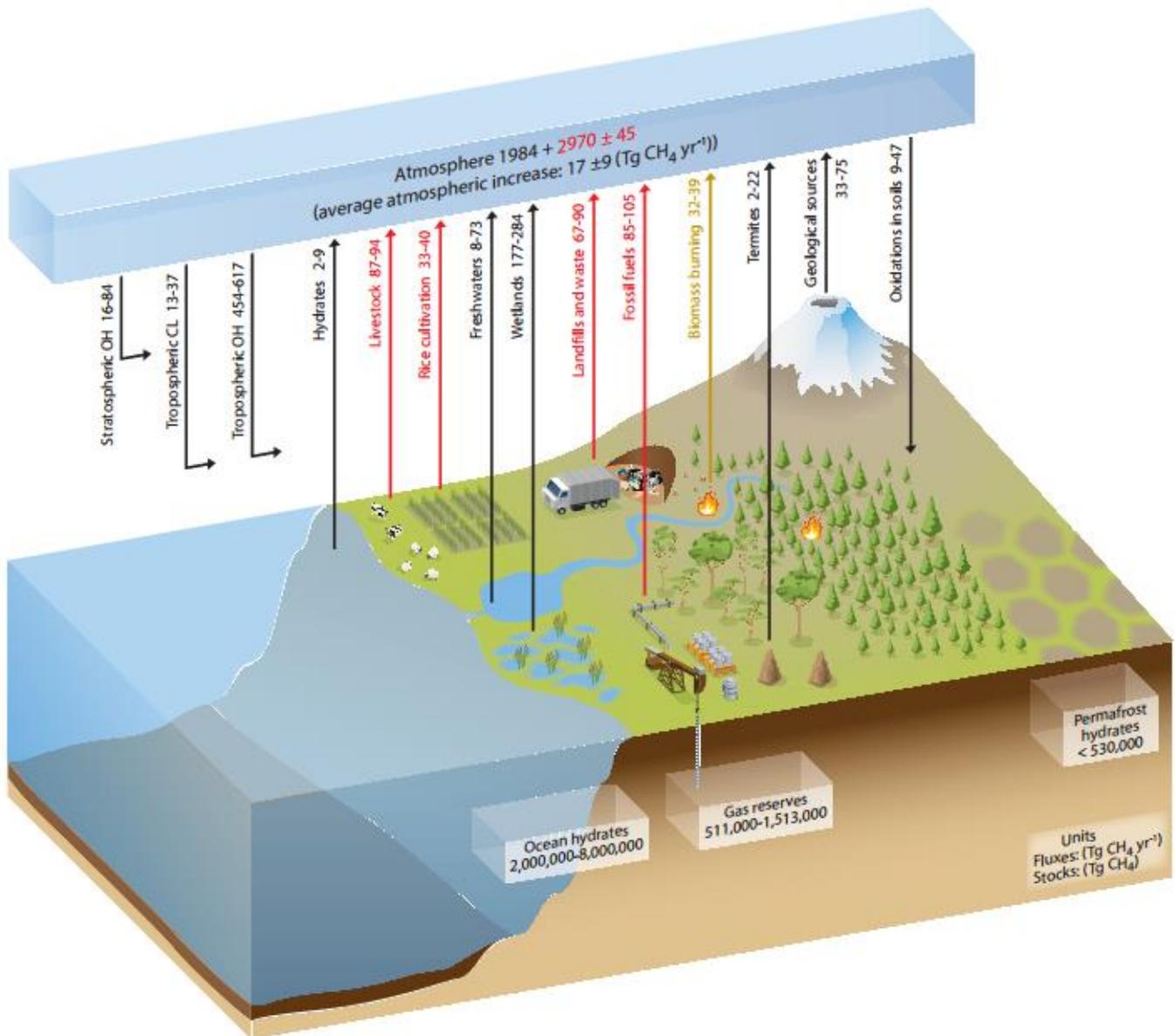


Figure 1. Schematic of the global cycle of CH₄ and average estimated contributions of different sources and sinks to the global CH₄ budget as presented in the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Ciais et al. 2013). Numbers represent annual fluxes in TgCH₄ yr⁻¹ estimated for the time period 2000-2009 and CH₄ reservoirs in TgCH₄: the atmosphere and three geological reservoirs (hydrates on land and in the ocean floor and gas reserves). Black arrows denote natural fluxes, red arrows anthropogenic fluxes, and the light brown arrow denotes a combined natural + anthropogenic flux. Ranges represent minimum and maximum values from cited references in Ciais et al. 2013.

Regarding natural sources of CH₄, natural emissions arise from wetlands, freshwaters (lakes and rivers), geological sources, termites and hydrates (Figure 1). Although wetlands cover 6-7% of the Earth's surface (Lehner and Döll, 2004), they play a dominant role in the global CH₄ budget, being the single largest natural source (Bridgham et al. 2013). The current emission estimates range from 177 to 284 TgCH₄ yr⁻¹, with mean value of 217 TgCH₄ yr⁻¹, which accounts for 35 to 50% of the mean total global emissions (Ciais et al. 2013) (Figure 1). Freshwaters (lakes and rivers) usually have not been mentioned in many global budgets but may represent another major source contributing 8-73 TgCH₄ yr⁻¹ (Ciais et al. 2013). However, it is important to note that anthropogenic sources are dominant over natural sources in multiple atmospheric CH₄ inversion models (top-down)¹ (~65%) but they are of the same magnitude when a different method (i.e. bottom-up models and inventories)² is used to estimate their contribution of the total global CH₄ emissions.

1.2. The Importance of inland waters on Methane Emissions

While boreal and subarctic inland aquatic environments store most of the global soil carbon stock, tropical and subtropical wetlands and freshwater environments are responsible for the majority (i.e. 47 to 89%) of global natural CH₄ emissions (Bridgham et al. 2013). Furthermore, there are indications that they emit up to 400% more CH₄ than similar environments in boreal and temperate biomes (Bastviken et al. 2010). Thus, during the last decades, several studies suggest that the CH₄ fluxes from anthropogenic sources were not, always, the primarily driven of the large increase and variation in CH₄ concentrations in the atmosphere (Bousquet et al. 2006;

¹ Top-down methods estimate regional CH₄ emissions by combining data on the global distribution of atmospheric CH₄ concentrations with spatial distribution of CH₄ sources and sinks.

² Bottom-up methods involve scaling CH₄ fluxes estimated with empirical ground-based (e.g. via chambers) or model-derived flux estimates and are used to attribute decadal budgets to individual processes emitting CH₄.

Kirschke et al. 2013). They indicate that climate driven fluctuations of CH₄ emissions from tropical natural wetlands were the main drivers of the global interannual variability of CH₄ emissions. For example, tropical wetland emissions are believed to explain the increased in the growth rate of atmospheric CH₄ that began in 2007, after nearly a decade of little growth (Kirschke et al. 2013). Despite being a recognized important global source of atmospheric CH₄, direct measurements of tropical wetlands and freshwaters are still scarce and the estimates are highly variable (Melton et al. 2013) (Table 1).

Table 1. Simulated annual mean total CH₄ emitted to atmosphere from natural wetlands for 1993-2004. All units are TgCH₄ yr⁻¹ ± 1σ, where the standard deviation represents the inter-annual variation in the model estimates. These estimates are resulted of The Wetland and Wetland CH₄ Inter-comparison of Models Project (WETCHIMP) investigation which simulate large-scale wetland characteristics and corresponding CH₄ emissions. Ten models participated, varied from simple to relatively complex, including models that varies in scale and methods to calculate wetland size and location. Adapted from Melton et al. 2013.

Model	Global	Tropics (30° S–30° N)	Extratropics (> 35° N) ^a
LPJ-Bern ^c	181 ± 15	106 ± 2	65 ± 13
CLM4Me	206 ± 6	134 ± 5	62 ± 6
DLEM	141 ± 11	85 ± 7	39 ± 3
IAP-RAS	164 ± 4	115 ± 2	43 ± 2
LPJ-WHyMe			27 ± 2
LPJ-WSL	174 ± 10	122 ± 7	42 ± 2
ORCHIDEE	264 ± 12	184 ± 11	71 ± 4
SDGVM	199 ± 5	135 ± 6	59 ± 3
Mean ± 1σ	190 ± 39	126 ± 31	51 ± 15

Previous investigations have been conducted in the tropics (Bastviken et al. 2010, 2011) but the emissions both within and across sites can vary several orders of magnitude depending on

local conditions such as hydrology, vegetation and climate. Among the important drivers of CH₄ dynamics in wetlands and tropical lakes the contribution of vegetation is arguably the least well understood (Carmichael et al. 2014).

Tropical wetlands, including coastal and freshwater lagoons, are among the most productive ecosystems on Earth (Esteves, 2011). These aquatic environments are generally shallow, allowing substantial development of the vegetated littoral zones (Esteves, 2011). The major CH₄-emitting sites are the littoral zones where emergent aquatic macrophytes dominate (Chanton et al. 1993) and participate in all three fundamental processes of CH₄ dynamics: production (Petruzzella et al. 2013), oxidation (Jespersen et al. 1998; Kankaala and Bergstrom, 2004) and, particularly, emission (Laanbroek, 2010). These processes vary greatly among plant species and life forms, and their net impact upon CH₄ emission can range from negative to positive (Ding et al. 2005; Koelbener et al. 2010; Bhullar et al. 2013).

Because of the substantial role that CH₄ plays on the Earth's climate system associated with, tropical wetlands and freshwaters are among the most prominent sources of unexplained spatial and temporal variability in global CH₄ emission estimates (Bousquet et al. 2006), it is essential to gain a better understanding of CH₄ dynamics in natural systems as well as the main factors that affect and regulating them, focusing mainly on the role of aquatic vegetation.

2. Methane Dynamics in Wetland and Freshwater Ecosystems

2.1. Methane Production

In wetland soils, under typical anaerobic conditions associated with the depletion of alternative terminal electron acceptors employed by the microbial community for the decomposition of organic matter, methanogenesis (i.e. CH₄ production) occurs as the final step of

the organic matter degradation (used in the following order: NO_3^- (denitrification) > Mn^{4+} (manganese reduction) > Fe^{3+} (iron reduction) > SO_4^{2-} (sulfate reduction) > CO_2 (methanogenesis)) (Neue et al. 1997; Grey box – Figure 2). Methanogenic *Archaea* gets energy and growth requirements by producing CH_4 , which is the result of complex of microbial activities that include both syntrophic interactions and competition for key substrates (Whalen, 2005) (Figure 2).

Organic carbon needs to be broken down to simple substrates before it can be utilized by methanogens. This process involves a consortium of specialized microorganisms that interact because none of these is capable to totally decomposing complex substrates (Whalen, 2005). Microbial exo-cellular enzymes decompose complex polymers to monomers such as, glucose and other simple sugars, followed by subsequent degradation steps by fermenting bacteria (Drake et al. 2009; Bridgham et al. 2013). Fermenting bacteria decompose monomeric compounds to alcohols, short chain fatty acids, CO_2 and Hydrogen gas (H_2). Thus, these end products are further utilized to produce acetate via acetogenesis by syntrophic bacteria or homoacetogenic bacteria. While syntrophic bacteria break down fatty acids and alcohols to acetate and CO_2 using H^+ as an electron acceptor to form H_2 , homoacetogenic bacteria are capable of degrading monomers like sugars directly to acetate. Only after this degradation process, fermentation end products (e.g. H_2/CO_2 and acetate) are available for methanogenesis (Whalen, 2005).

There are two main ways by which CH_4 is generated by methanogens as a metabolite in energy production (Whalen, 2005). Acetotrophic methanogens use acetate as a substrate to produce CH_4 and CO_2 gases:

Acetoclastic Methanogenesis:



while hydrogenotrophic methanogens act as living electron acceptors, reducing CO_2 to CH_4 with electrons provided via interspecies H_2 transfer (syntrophic bacterias):

Hydrogenotrophic Methanogenesis:



Although only 14% of methanogenic species can use acetate as a carbon and energy source, acetoclastic methanogenesis (Equation 1.1) is considered responsible for two-thirds of CH_4 produced (Le Mer and Roger, 2001) and, generally, is favored in environments with abundant labile organic carbon. In contrast, about 77% of methanogenic species are hydrogenotrophic (Equation 1.2) and this pathway predominates in the more recalcitrant layers (Hornibrook et al. 1997). Despite of H_2 - CO_2 and acetate are primary substrates for methanogenesis, other substrates (e.g. methanol, methylamines, formate) can be utilized by some more versatile methanogens species (e.g. methanosarcinaceae) (Zinder, 1993).

2.2. Methane Oxidation

Only part of the CH_4 produced is emitted to the atmosphere. Considerable amounts are consumed by methanotrophs, group of microorganisms that use CH_4 as their only carbon and energy source (Segers, 1998) (Figure 2). Two forms of CH_4 oxidation are recognized in soils: the first is named high affinity oxidation, which occurs at CH_4 concentrations close to that of the atmosphere, and it is estimated to contribute 10% of the total CH_4 consumption (Le Mer and Roger, 2001). The second known as low affinity oxidation, it takes place at high CH_4 concentrations and is considered methanotrophic activity *sensu stricto* (Segers, 1998).

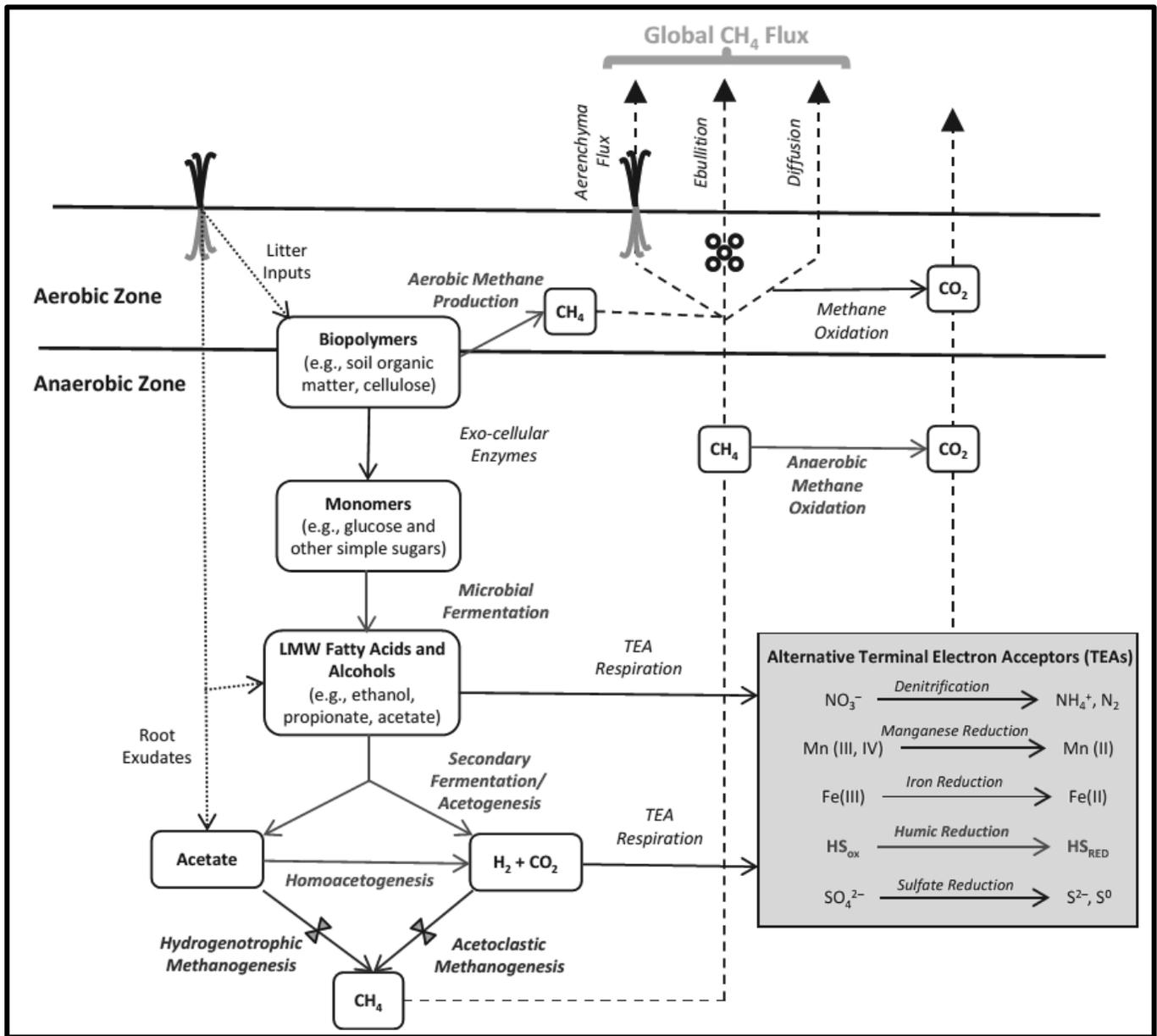
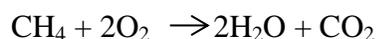


Figure 2. CH₄ cycling in wetland ecosystems. Pools of carbon are shown in boxes and solid arrows show the progressive mineralization of these carbon pools by the identified microbial processes or groups. Dotted lines illustrate carbon inputs from emergent aquatic macrophytes. Dashed lines represent the flux of the gaseous end products of these processes (CH₄ and CO₂) to the atmosphere (from Bridgman et al. 2013).

Oxygen availability appears to be the main factor limiting methanotrophy. First, the enzyme responsible for catalyzing the initial step in the oxidative pathway (methane monooxygenase) can break the O-O bonds of oxygen molecules, reducing one oxygen atom to H₂O and the other to CH₃OH (methanol) by incorporating CH₄. Second, oxygen serves as a terminal electron acceptor in CH₄ oxidation resulting in CO₂ (Whalen, 2005) (Equation 1.3).



Equation 1.3

In wetlands, methanotrophs develop in the thin oxidized soil layer, in the water column and particularly, in the aerobic rhizosphere of aquatic plants (Laanbroek, 2010). Previous studies estimated that these organisms can consume 43-90% of the CH₄ produced in aerobic soils (Le Mer and Roger, 2001). As a result, methanotrophic bacteria can limit the amount the CH₄ that is released to the atmosphere substantially.

2.3. Methane Transport

CH₄ can be emitted from aquatic systems through several different pathways, including plant-mediated transport, ebullition and diffusion (Figure 2) and the relative contribution of these various routes differed substantially between wetland types (Bastviken et al. 2011; Tokida et al. 2013; Hamilton et al. 2014). For example, Bastviken et al. (2010) measured CH₄ emissions from 16 natural shallow lakes during the low water period in the Pantanal region of South America and found that ebullition accounted for more than 90% of the CH₄ emitted. Hamilton et al. (2014) suggest that tropical floodplains with inundation depths > 1m have low rates of CH₄ emission via rooted emergent and floating-leaf plants, in contrast to results from rice fields and temperate

wetlands in which plant-mediated transport dominates even over these depths (Bergstrom et al. 2007). In order that, several studies have been demonstrated that plant-mediated transport tends to predominate in shallow waters containing rooted emergent plants (Laanbroek, 2010; Bridgham et al. 2013) but in sparse stands and unvegetated areas bubbling and diffusion are more important mechanisms of CH₄ transport (Kankaala et al. 2004; Bergstrom et al. 2007).

The mere presence or absence of vegetation can drastically impacts the magnitude of CH₄ flux from sediment to the atmosphere (Schimel, 1995; Bergstrom et al. 2007). Sorrell and Boon (1994) investigated the importance of lacunar gas transport from a zone dominated by *Eleocharis sp. phacelata* compared with an unvegetated zone in a freshwater wetland in Australia and was found 1-15 times higher CH₄ emission rates from macrophyte stands. In an arctic wet meadow, the removal of sedges led to reduced CH₄ emissions and increased accumulation of CH₄ in flooded soils (Verville et al. 1998). According to some estimates, the contribution of plant-mediated CH₄ to the atmosphere can be very variable and depends on the vegetation type, ranging from 30 to 100% of the total CH₄ flux (Bridgham et al. 2013). Emergent aquatic macrophytes' roots and rhizomes form a permanent connection that acts as a conduit between sediment and atmosphere, providing oxygen to their underground parts. Following the opposite pathway, CH₄ produced in the sediment enters the roots and is transported directly to the atmosphere (see Laanbroek 2010 for a review).

There are two major mechanisms involved in plant-mediated transport of CH₄ from freshwater wetlands to the atmosphere, namely molecular diffusion and convective gas flow. Molecular diffusion is a passive transport mechanism driven by the respiratory uptake of oxygen by plants, which creates a diffusion gradient that transports oxygen from the atmosphere to the roots and rhizomes. This is accompanied by an upward diffusion of CH₄ from the rhizosphere to

the atmosphere via aerenchyma following the concentration gradient (Brix et al. 1992). On the other hand, internal pressurization is the driving force behind the convective gas flow, which is based predominantly on differences in humidity and temperature between the plant aerenchyma and external atmosphere (Armstrong et al. 1992). These differences generate a pressure gradient that drives gas flow from leaves to rhizome and then vent back to the atmosphere through old leaves (Brix et al. 1992). This allows greater rhizome and root growth, especially in anoxic flooded soils, and permits growth in deeper water (Sorrell and Hawes, 2010). This mechanism obtains much greater rates of internal oxygen transport than does simple diffusion, increasing internal oxygen concentrations in underground rhizomes, as well as root oxygen release and rhizosphere oxidation (Armstrong et al. 1996).

3. Factors Affecting Methane Emissions: The role of Vegetation

While several studies have found CH₄ emissions to the atmosphere to be increased by the presence of aquatic macrophytes (see Laanbroek, 2010 for a review), others have found them to be decreased (Ström et al. 2005). These contradictory results may partly be attributed to the environmental conditions under which the studies were performed. Thus, it is important to note that CH₄ emissions from wetlands is a complex process that is controlled by multiple factors besides that mentioned below, such as water table, temperature, pH, nutrients and soil type (Christensen et al. 2003; Lai, 2009) which of these topics was not discussed in this dissertation.

3.1. Supply of Organic Substrate

The supply of organic substrate is a master control on methanogenesis once anaerobic condition is established and terminal alternative electron acceptors are depleted (Conrad, 2007). In this way, aquatic macrophytes can stimulate methanogenesis increasing carbon substrate availability by allocation of organic compounds through root exudation and the quality and

quantity of plant litter, which can the former account for 50% of the nutrients and organic matter input in an entire aquatic system (Wetzel, 2001; Laanbroek, 2010; Dorodnikov et al. 2011). In addition, Farjalla et al. (1999) noted that the plant litter, can be more resistant to decomposition, such as emergent macrophytes, or be more labile, such as floating leaves or submerged macrophytes.

A large amount of carbon assimilated by these plants through photosynthesis can be allocated belowground. Once released to the soil, these rapidly decomposable carbon pools can serve as substrate for fermenting bacteria but, however, there is no agreement about their actual contribution for methanogenesis (Koelbener et al. 2010; Dorodnikov et al. 2011). Ström et al. (2003) showed that the acetate formation rate was much higher in *Eriophorum scheuchzeri* microcosms in control than in shaded plots, indicating that higher photosynthetic rates in control plots leads to higher allocation of carbon to the root zone and, subsequently, to higher acetate formation, affecting substrate quality and influence CH₄ formation.

On the other hand, several studies also suggest that products of photosynthesis made a very limited contribution under not carbon limiting conditions (Dorodnikov et al. 2011; Bhullar et al. 2014). Dorodnikov et al. (2011) using ¹⁴C-labelling experiment have shown that the contribution of recent photosynthates to methanogenesis in different peatlands' vegetation types accounted for 0.03% for *Eriophorum vaginatum* from elevated hummocks, 0.06% for *Eriophorum vaginatum* from laws and 0.13% for *Scheuchzeria palustris* from wet hollows of assimilated ¹⁴C. Additionally, King and Reeburgh (2002) and King et al. (2002) also demonstrated in mesocosms of moist tussock and wet sedge tundra that only 0.05 to 5% of photosynthesized carbon contributed to CH₄ emitted. Nutrient availability, in particular

phosphorus availability also determines the quantity of root exudates (Koelbener et al. 2010; Bhullar et al. 2014).

3.2. Plant Primary Productivity

Several studies have reported a positive relationship between plant productivity and CH₄ emissions from flooded sediments (Chanton et al. 1993; Whiting and Chanton, 1993). Whiting and Chanton (1993) observed this positive relationship across a range of wetlands with a water table close to or above the surface, presumably because a higher net productivity leads to, as mentioned before, a higher input of labile carbon substrate for methanogenesis and increased gas transport rates by aquatic plants (Laanbroek, 2010). Additionally, Joabsson and Christensen (2001) demonstrated a higher CH₄ flux from control than from shaded plots (with artificially reduced net productivity) in a Greenland wet tundra dominated by *Eriophorum* and *Carex* in the Zackenberg wetland. However, some studies have found either a negative or no relationship between productivity of vegetation and CH₄ emission (Ström et al. 2005; Bouchard et al. 2007). It is important to note, however, when evaluated at the plant species level, the relationship between biomass and CH₄ flux becomes less predictable. As mentioned above, Joabsson and Christensen (2001) showed higher rates of CH₄ with greater plant biomass for some species but no correlation was found with the biomass of another dominating sedge *Dupontia psilosantha*. Furthermore, a comparison of three species derived from the same habitat also indicated that plant productivity was not a predictor of CH₄ fluxes (Ström et al. 2005). Instead, differences in CH₄ oxidation rates among the three species better explained CH₄ flux.

3.3. Vegetation Type

Vegetation type is a key factor influencing wetland and freshwaters CH₄ emissions. Because of their permanent access to the atmosphere in combination with their morphological

adaptations to a life under oxygen-limited conditions as mentioned before, emergent aquatic macrophytes are the greatest emitters (Bergstrom et al. 2007). Bergstrom et al. (2007) investigated the role of vegetated littoral area in the efflux of CH₄ in 619 lakes in Finland and found that the natural open ombrogenous bogs and minerogenous fens covered a 2.5-fold larger area than the littoral zones with emergent aquatic macrophytes, but their emissions were estimated to be only 78% of the emissions by *Phragmites australis* and *Equisetum fluviatile*, the two most dominant species in the area. The zone floating-leaved-dominated, *Nuphar lutea*, *Potamogeton natans* and *Sparganium* spp. covered 44% of all vegetated littoral areas, but their contribution was approximately 2% of the dominant emergent macrophytes, indicating the superiority of this life form. Additionally, Duan et al. 2005 reported that the average emission flux of CH₄ from submerged macrophyte (*Potamogeton pectinatus*) growing zones was 85.8% lower than from emergent macrophyte (*Phragmites australis*) growing zones.

3.4. Herbivory

An emerging viewpoint regarding the role of the aquatic macrophytes in freshwater wetlands CH₄ emissions is the influence of herbivory on CH₄ dynamics. Some studies have evaluated the potential impact of herbivorous on CH₄ emissions from aquatic macrophytes using artificial manipulation (e.g. clipping) (Schimel, 1995; Greenup et al. 2000; Ding et al. 2005; Cheng et al. 2007) and under natural field conditions (Bodelier et al. 2006; Dingemans et al. 2011). There are two studies that addressed the herbivory impact on CH₄ emissions in natural conditions, Bodelier et al. (2006) showed that swan grazing on belowground tubers of a submerged macrophyte had both direct and indirect impacts on CH₄ flux, one of these was the reduction of CH₄ production activity of methanogenic microorganisms. In a more recent study, Dingemans et al. (2011)

reported the herbivorous birds grazing impact on CH₄ emissions from emergent aquatic macrophytes and found 5 times higher CH₄ flux rates than control plots without grazing.

Clipping of shoots of emergent macrophytes has generally been used to demonstrate the transport of oxygen into the rhizosphere and, in the opposite way, the facilitation of CH₄ release from the sediment to the atmosphere (Laanbroek, 2010). Notably, the effect of herbivore damage resembling clipping. Some studies indicated that the increased of CH₄ fluxes in clipped treatments or in the plots of severed vegetation in the field could be result from a decreased resistance to diffusion of CH₄ (Schimel, 1995; Kelker and Chanton, 1997; Cheng et al. 2007; Dingemans et al. 2011).

Thus, acknowledging that plant species differ in important features which influence gas transport. Additionally, these differences consequently affect the underlying microbial processes which depend on their oxygen and carbon release (Laanbroek, 2010), little is known about the role of herbivorous play on plant-mediated CH₄ emissions, especially by insects, despite the large numbers of herbivorous insects present in tropical freshwater wetlands globally.

4. General Objective

In order to bridge the gap between the herbivory caused by insects and their effect on plant-mediated CH₄ emission, the aim of this dissertation is to verify if insect damage affects the CH₄ emissions by *Eleocharis equisetoides*, an emergent macrophyte specie.

For that, we performed a mesocosm experiment in which we simulated the damage caused by herbivorous insects and manipulated the density of damaged culms of *Eleocharis equisetoides* (4 levels – 0, 20, 50 and 100%) measuring the corresponding CH₄ flux, concentration and potential CH₄ production in the sediment (Chapter 1)

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CHAPTER 1

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Does herbivore damage increase methane emissions from emergent aquatic macrophytes?

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Abstract

Wetlands are the single largest natural methane (CH₄) source and the vegetated littoral areas are the major contributors to release them to the atmosphere. Although the effects of herbivores on emergent aquatic macrophytes have been well documented, their effect on plant-mediated CH₄ fluxes, especially by insects, remains unknown. We performed a mesocosm experiment in which we simulated the damage caused by herbivorous insects and manipulated the density of damaged culms of *Eleocharis equisetoides* (4 levels – 0, 20, 50 and 100%) measuring the corresponding CH₄ flux, concentration and potential CH₄ production in the sediment. We hypothesized that an increased percentage of plants with simulated herbivory would be associated with increased CH₄ flux rates. Simulated herbivory positively affected CH₄ emissions, but only under high herbivory pressure. The average CH₄ flux from 100% damaged plants mesocosms was 3.5 higher than mesocosms with all intact plants (0% damage). These results indicate that physical damage on emergent macrophytes affects gas transport within the plants. A field survey in our studied system revealed that plant biomass consumed by herbivores is relatively low compared to the actual number of damaged plant culms. This particular result highlights the fact that herbivorous insects may have a disproportional effect on CH₄ emissions, due to physical damage, compared to their effect on plant biomass loss. In summary, our findings bring a new perspective to the influence of herbivory on the CH₄ and carbon cycling, especially regarding the role that herbivorous insects might play.

Keywords: Herbivory; Coastal lagoons; Carbon cycle; Gas transport; Methanogenesis.

1. Introduction

Among global ecosystems, wetlands are one of the most important organic matter (OM) degradation sites, and are the largest natural source of atmospheric methane (CH_4) (Bridgham et al. 2013). Average CH_4 emissions from wetlands are 217 Tg yr^{-1} (Ciais et al. 2013). Within wetlands, the littoral zones are major sources of CH_4 emissions. Aquatic macrophytes dominate the littoral zone (Bergstrom et al. 2007) and participate in all three fundamental processes of CH_4 dynamics: production (Petruzzella et al. 2013), oxidation (Jespersen et al. 1998) and, particularly, emission (Laanbroek, 2010).

The contribution of plant-mediated CH_4 to the atmosphere can be very variable, ranging from 30 to 100% of the total CH_4 flux (Bridgham et al. 2013). Several studies have shown that, compared with other life forms (e.g., floating-leaved and submerged), emergent aquatic macrophytes are the greatest contributors to CH_4 fluxes from wetlands (Duan et al. 2005; Bergstrom et al. 2007). However, CH_4 emission rates by emergent macrophytes are influenced by a variety of factors, including sediment CH_4 concentration and production (Ding et al. 2003); the presence or absence of morphological features (aerenchyma structures) (Sorrell and Boon, 1994); the mechanisms by which gas flows within aerenchyma tissues (Dacey, 1980; Armstrong et al. 1992; Brix et al. 1992) and, recently investigated, herbivory (Dingemans et al. 2011).

Emergent aquatic macrophytes are adapted to oxygen-limited conditions. Their roots and rhizomes form a permanent connection that acts as a conduit between sediment and atmosphere, providing oxygen to their underground parts. Following the opposite pathway, CH_4 produced in the sediment enters the roots and is transported directly to the atmosphere (see Laanbroek, 2010 for a review). Thus, any physical harm to the plants could affect the rate or the magnitude of gas transport, as shown in some previous studies (Ding et al. 2003, 2005). Studying the effects of

herbivorous birds on aquatic macrophytes, Dingemans et al. (2011) demonstrated that CH₄ emissions from stands subjected to herbivory could increase approximately 5 times compared with non-affected macrophyte stands. However, plants species vary in their capacity to transport CH₄ from the sediment to the atmosphere (Bhullar et al. 2013). Because their study focused on only one plant-bird interaction, a research priority is to assess grazing effects on other plant species and by other herbivorous species. Additionally, artificial and natural shoot clipping experiments have generally been used to demonstrate gas transport through macrophytes (Ding et al. 2005; Laanbroek, 2010; Dingemans et al. 2011). Despite the well-documented effects of herbivores on aquatic macrophytes (Van Eerden et al. 1997; van den Wyngaert et al. 2003; Gauthier et al. 2005; Hidding et al. 2009), the effect of herbivory, and especially by insects, on potential CH₄ emissions has not been studied.

Studying biomass production of aquatic macrophytes in tropical coastal lagoons, dos Santos and Esteves (2002) measured herbivory by grasshoppers (Order: Orthoptera), which usually use aquatic plants as a food source, shelter from predators, oviposition and nymph development sites in wetlands. They found that the net annual aboveground primary production loss due to insect herbivory in *Eleocharis interstincta* (Vahl) Roem. and Schult. was relatively low ($\approx 2\%$). However, they observed that plants were always damaged in the apical part of the culms, above the water line, and that half (longitudinal section) of the culm was consumed. The form of damage caused by grasshoppers did not reduce the culms' survival because only the tips are damaged. Thus, damaged culms can act as an open conduit for CH₄ release to the atmosphere, potentially resulting in higher CH₄ emission rates.

While there are indications that tropical lakes and reservoirs emit up to 400% more CH₄ than similar environments in boreal and temperate biomes, direct measurements are still scarce

(Bastviken et al. 2010). Previous investigations have been conducted in the tropics (Bastviken et al. 2011), but the contribution of vegetation to the CH₄ emissions is arguably the least well understood (Carmichael et al. 2014). Despite the potential importance of herbivory in modulating CH₄ emission rates (Schimel, 1995; Kelker and Chanton 1997; Dingemans et al. 2011), any study was developed to examine the role that herbivorous insects might play in the CH₄ dynamics. Understand these insect-plant interactions could be very important, mainly, in highly vegetated tropical ecosystems such as shallow lakes and coastal lagoons, which suffers high herbivory pressure (Cyr and Pace, 1993; Lodge et al. 1998; Franceschini et al. 2010).

E. equisetoides is abundant in many coastal lagoons and inland lakes in the tropics (Kissmann and Groth, 1997) and is one of the main components of the orthopteran diet in these ecosystems (dos Santos and Esteves, 2002). The presence of grasshoppers of the genus *Stenacris* (Family: Acrididae) are recorded in our study system. Biological information on *Stenacris* is scanty but they appeared throughout the year, as well as damaged culms of *E. equisetoides* (A. R. Gripp, Personal communication; dos Santos and Esteves, 2002). The exception of their occurrence is during the period of new culm growth after occasional sandbar breaching, indicating great influence by water level fluctuations on their population dynamics (Capello et al. 2013).

In the present experiment, we used an experimental approach within mesocosms to simulate herbivory. We manipulated the percentage of damaged plants using the aquatic macrophyte *Eleocharis equisetoides* (Elliott.) Torr. and measured the corresponding CH₄ flux, concentration and potential CH₄ production (PMP) in the sediment. Thus, we hypothesized that an increased percentage of plants with damage from simulated herbivory would be associated with increased CH₄ fluxes.

2. Methods

2.1. Experimental design

The mesocosm experiment was conducted at Núcleo em Ecologia e Desenvolvimento Socioambiental de Macaé (NUPEM/UFRJ - Macaé, Rio de Janeiro, Brazil; 22°19'S, 41°44'W). The mesocosms were constructed using plastic cylinders (27 cm diameter and 45 cm deep). Sand, water and emergent aquatic macrophyte shoots (*E. equisetoides*) were taken from the Jurubatiba lagoon. We also sampled organic sediment from Amarra Boi lagoon, located near Jurubatiba lagoon. Both lagoons are in Restinga de Jurubatiba National Park (22°-22°30'S and 41°15'-42°W), but Jurubatiba lagoon has an elongated and ramified shape with an extensive littoral zone. We used organic sediment from different lagoons because of the high OM content of the Amarra Boi lagoon (~ 74%). A previous experimental assay demonstrated that the mesocosms produced no detectable CH₄ emission. This was probably due to the low availability of labile organic carbon in the sediment. We added a natural substrate to ensure that the system was not carbon limited. We also added leaf fragments from aquatic macrophytes (30 g wet weight per mesocosm) to the mesocosms' sediment to increase OM input and create an anoxic environment; stimulating CH₄ production. This also mimics the natural input of OM observed along the margins of coastal lagoons. Final average OM as a percentage of the sediment in the experimental mesocosms was approximately 6%.

We established twenty-four mesocosms in an open field and exposed them to full sunlight and ambient temperatures and precipitation (Fig. 1). Each mesocosm was filled with 8550 cm³ of a homogenized mixture of sand and organic sediment using a 10:3 ratio, followed by a second layer of organic sediment (1150 cm³); five plant shoots were carefully transplanted into the mesocosms. At the time of transplanting, shoots had different numbers of culms, but there was no

difference in the number of *E. equisetoides* culms ($n= 44$ in average) among mesocosms after a three-month growth period (ANOVA $F_{3,20}=1.04$, $P=0.3$; $n=24$). The mesocosms were watered with well water maintained at a depth of 6.5 cm during the whole experiment.

We assigned the twenty-four mesocosms to four treatments with six replicates each. We manipulated the percentage of damaged plants in the mesocosms as follows: (1) 0% (all plants intact), (2) 20% (damage to twenty percent of plants), (3) 50% (damage to fifty percent of plants), and (4) 100% (damage to all plants). Treatment units were rested for three months after the initial transplants. We used a razor blade to simulate 5-10 cm of damage on the plants of each corresponding treatment level (except 0%). The simulated plant damage was similar to the damage caused by herbivorous grasshoppers of the order Orthoptera (Fig. 2). dos Santos and Esteves (2002) reported that *Eleocharis* is grazed mostly by this organism. The damage matched what we observed in natural fields.

2.2. CH₄ measurements

The measurements were made two weeks after the simulation of grasshopper damage. CH₄ flux rates were measured using a static closed chamber as described by Denmead (2008). To capture the entire CH₄ flux, the acrylic chamber (28 cm diameter and 24.2 cm height) was placed over each mesocosm and sealed with a rubber gasket. We carefully sampled each mesocosm to minimize soil disturbance, avoiding ebullition. Air inside the chambers was circulated with a battery-driven fan during the measurements to ensure that the gas samples were well mixed. An initial sample was collected, after which gas samples were taken from the headspace of the chamber every 3 minutes during a 9 minutes sampling period ($n = 4$), according to Kankaala (2004a,b). Samples were collected with 3 mL plastic syringes. CH₄ fluxes were calculated as: $F = (S \cdot V/A) \cdot T$ where F is the CH₄ flux ($\text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), S is the slope of the linear regression

between CH₄ concentrations (ppm) in the static chamber headspace against time of sampling (Appendix S1), V the volume of the chamber (L), A the area of the chamber (m²) and T is the time (h). The slopes from linear regressions were rejected when r^2 was less than 0.65 (Appendix S1). The CH₄ concentration in the gas samples was measured with gas chromatography (VARIAN Star 3400 – Varian Co., USA and Shimadzu - 2010), with a FID detector temperature of 220°C, an injector temperature of 120°C, a 1 m Poropak-Q column (60/100 mesh) at 85°C and N₂ as the carrier gas.

Additionally, we measured the sediment CH₄ concentration and PMP in the mesocosms. To determine the sediment CH₄ concentration, two samples (5 mL each) were collected with a plastic syringe, and the results were averaged for each mesocosm. The samples were stored in 25 mL flasks with 5 mL NaCl supersaturated solution to expel the CH₄ from the pore water. The flasks were closed with rubber stoppers and shaken. The CH₄ concentration was analyzed by gas chromatography. Two samples (5 mL each), averaged within a mesocosm, of the sediment were collected and transferred to 25 mL flasks to determine PMP; 5 mL of water from the mesocosm was added. The flasks were closed with rubber stoppers, and the residual CH₄ and O₂ were removed by flushing with N₂ for 1 min, creating an anoxic and CH₄-free condition. The flasks were incubated statically in the dark at a constant temperature (25±0.5°C) for three days. After the incubation period, the CH₄ concentration in the flasks' headspace was measured with gas chromatography.

2.3. Field measurements

To estimate the number of *E. equisetoides* culms damaged by herbivores and the effect on plant biomass in natural conditions, we performed a field sampling during the experiment. We sampled and harvested 10 randomly selected *E. equisetoides* quadrats at the Jurubatiba lagoon;

quadrats were 0.0625 m². Field samples were taken to the laboratory. For each quadrat, we determined the proportion of damaged and nondamaged culms. We oven-dried individual culms at 60°C to constant weight and then determined their dry biomass. To calculate the percentage (%) of culms damaged by herbivores, we counted the damaged culms and divided by the total number of culms per quadrat. To calculate the biomass loss, first, we regressed the plant length (cm) with dry weight (g) for intact and damaged individuals (Appendix S2). The aim of this procedure was to obtain a linear equation for each group (i.e., intact and damaged culms). We tested the homogeneity of the slopes for both equations and found no significant difference (ANCOVA $P=0.17$). To estimate the biomass loss due to herbivory (BR), we used an average culm length of all sampled plants in the linear equations. We then subtracted the resulting estimated biomass of damaged culms from that of intact culms. We used the estimated values from linear equations to control for individual culms length differences. Second, to estimate the total biomass per quadrat in the absence of herbivory (BT), we summed the biomass of all intact culms (BI) and the biomass of damaged culms (BD) with BR ($BT= BI + (BD + BR)$). The average percentage (%) biomass loss was calculated from the ratio BR:BT.

2.4. Statistical analyses

Relationships between percentage of damaged plants and CH₄ fluxes, CH₄ sediment concentration and potential production were evaluated using an one-way analysis of variance (ANOVA). Variation in these response variables among levels of our predictor variable (i.e., percentage of damaged plants - 0, 20, 50 and 100%), was tested using orthogonal planned contrast, as a post-hoc test (Scheiner and Gurevitch, 2001). For the analysis, 50% (twelve of 24) of the mesocosm results were rejected because did not met the $r^2 > 0.65$ linearity criteria

(Appendix S1). Therefore, we only used in data analysis these 12 mesocosms for all remaining response variables.

Prior to data analysis, data, if necessary, was subjected to log transformation to meet the criteria of normality and homogeneity of variance. All statistical analyses were conducted with R software version 3.02 (R Core Team, 2014).

3. Results

Simulated plant damage affected CH₄ emissions (Fig. 3A). *E. equisetoides* mesocosm fluxes increased in response to the percentage of damaged culms ($F_{3,8}=5.02$, $P =0.03$; $n=12$), ranging from 0.04 to 0.35 mg. m⁻². h⁻¹, for 0% and 100% damaged plants treatments respectively. The average CH₄ flux from 100% treatment was 3.5 higher than the average from mesocosms with all intact plants (0%). However, 0% and 20% treatments were not significantly different ($t=0.45$, $P =0.66$), as well as 50% and 100% damaged plants treatments ($t=0.12$, $P =0.90$), but significant difference was found between the lowest (0 and 20%) and the highest simulated damaged levels (50 and 100%) ($t=-2.88$, $P =0.02$).

The percentage of damaged plants had no significant effect on the sediment CH₄ concentration ($F_{3,8}=1.64$, $P =0.25$; $n=12$) (Fig. 3B) and PMP measurements ($F_{3,8}=0.94$, $P =0.46$; $n=12$) (Fig. 3C).

Field assessments of biomass loss due to grasshopper herbivory revealed that only 4.18% of plant biomass was consumed. This represents damage to approximately 15.2%, per square meter, of culms in the *E. equisetoides* stands in the field.

4. Discussion

Emergent aquatic macrophytes can influence CH₄ emissions from wetlands by altering processes such as production, consumption and transport of CH₄ to the atmosphere (Ding et al. 2005; Ström et al. 2005; Laanbroek, 2010). Wetland plants are often damaged by herbivory, with resultant biomass loss. This damage may have specific effects on CH₄ dynamics; effects on CH₄ emission rates and sediment processes are debated in the literature (Schimel, 1995; Greenup et al. 2000; Bodelier et al. 2006; Dingemans et al. 2011) but the role that herbivorous insects play on this dynamic has never been investigated. In this study, we found that CH₄ fluxes were positively affected by the number of damaged plants but only under high herbivory pressure (50% and 100% damage). However, CH₄ concentrations and PMP in sediment were not affected. Corroborating with previous studies (Dingemans et al. 2011), these results indicate that physical damage on emergent macrophytes affects gas transport within the plants, rather than on CH₄ cycling by microbial processes. These findings bring a new perspective to the influence of herbivory on the CH₄ and carbon cycles, especially concerned with the role that herbivorous insects might play. Tropical ecosystems are composed, usually, by highly vegetated aquatic systems (Scheffer, 1998) and vegetation-mediated pathways are recognizably important (Bergstrom et al. 2007; Laanbroek, 2010), in this way, herbivorous insects might have an important role on CH₄ emissions.

Our experiment supports our hypothesis that induced plant damage has a positive effect on CH₄ emissions. Few studies have reported herbivory effects on CH₄ emissions, and their findings apparently are in agreement regarding the positive effects of damage caused by herbivores on CH₄ fluxes. Ding and coworkers (2005), studying CH₄ transport capacity in emergent plants from freshwater marshes, observed that all fluxes increased after plants were

clipped 3 cm above the water surface. Kelker and Chanton (1997) revealed that the CH₄ fluxes from clipped *Carex* plots were higher than unclipped about 97% to 111%. We observed CH₄ fluxes 3.5 times higher in average than undamaged plants mesocosms, i.e. treatments where all plants were intact. On the other hand, when only 20% of the culms were damaged, we observed no significant difference from mesocosms with 0% damage. This result is more consistent with those of Greenup et al. 2000, who found no significant difference in CH₄ fluxes between clipped and non-clipped *Eriophorum vaginatum* L. plots cut above the water table. We believe, based on low CH₄ fluxes emitted from 0% and 20% damaged plants mesocosms, that the flux contribution of individual culms is very low. Therefore, only when 50% and 100% of the plants were damaged, statistical differences were detectable.

The magnitude of CH₄ emissions from aquatic ecosystems may depend on sediment CH₄ concentrations, the nature of the damage and species-specific plant dynamics (Schimel, 1995; Laanbroek, 2010). Plant species vary in their capacity to transport CH₄ from the rhizosphere to the atmosphere because they possess different traits (Bhullar et al. 2013). According to Brix et al. (1992), CH₄ fluxes are directly proportional to diffusive resistance. We believe that the effect of herbivores on plant culms is strictly physical, reducing diffusive resistance by opening a free pathway for CH₄ outflow. Furthermore, it has been shown that quantitative effects of herbivores on CH₄ emissions depend strongly on whether grazing occurs below or above the water surface (Cheng et al. 2007; Dingemans et al. 2011). If grazing occurs above the water surface, CH₄ emissions are higher because CH₄ is not oxidized in the water column (King, 1992). Therefore, herbivory by orthopterans on emergent macrophytes, as measured in the present experiment, can be especially important because it only occurs above water level. In addition, herbivory damage to *E. equisetoides* culms does not necessarily cause plant death and is permanent; there is no

shoot regrowth after damage (dos Santos and Esteves, 2002), which may enhance herbivory effects on CH₄ emissions during the entire plant life cycle.

A field survey was performed in our study system to assess the biomass loss due to grasshopper herbivory and the amount of culms that are actually damaged. The field measurements revealed that only 4.18% of plant biomass is consumed. This represents, approximately, 15.2% of damaged culms in *E. equisetoides* stands. Our experiment results suggest that herbivory caused by insects at least at this particular sampling, would not significantly affect CH₄ emissions, assuming that mesocosms with 20% damaged plants was not different from 0% damage. However, this not means that the herbivory by grasshoppers is ecologically unimportant. At small spatial scales, factors that determine location selection by insects, for example, predation risk (Power and Rainey, 2000) or hydrology (Batzer and Wissinger, 1996), may indirectly affect CH₄ emissions because habitat use determine the grazing impact on emergent macrophytes (Salski and Holsten, 2009). In addition, the data regarding grasshoppers' population and feeding dynamics (e.g. seasonality) in our study system are very limited, and more studies are needed to evaluate the maximum herbivory pressure that these systems can actually suffers. It is important to note that the amount of biomass removed per square meter in a macrophyte stand is much lower that the number of damaged plants. This is expected due to how herbivorous insects consume this specific species of macrophyte. This particular result highlights the fact that herbivorous insects may have a disproportional effect on CH₄ emissions compared to their effect on plant biomass loss. As we showed in our experiment, a very small damage (i.e., low biomass removal), but when performed in many culms (50% and 100% of damaged plants treatments), significantly increases CH₄ fluxes. Recent studies started to recognize the potential importance of insect activities on CH₄ emissions, and the form of

herbivory and how the plant tissues are damaged can be an indirect sign to support this evidence (Carmichael et al. 2014).

Our results showed no significant effect between CH₄ concentration and PMP in the sediment and the percentage of damaged plants. Ding et al. (2005) observed that CH₄ emissions and pore water CH₄ concentrations both increased significantly in marshes with *Carex lasiocarpa* Ehrh plants after they were cut off above the water surface. This result may sound counterintuitive because the diffusive gas flows are maintained after clipping the culms above the water level, and O₂ can reduce methanogenesis and stimulate CH₄ oxidation (Laanbroek, 2010). However, the increase in CH₄ concentration when plants are damaged could have resulted from a reduction in the amount of O₂ transport to the roots. Although it is not possible to disentangle the importance of each mechanism involved in transporting gases from wetland plants, the efficiency of the mechanisms can be modified when aerial parts are clipped (Armstrong et al. 1996), affecting CH₄ and O₂ flow rates. Thus, O₂ transport to submerged parts can be reduced, and CH₄ may continually flow from the sediment as damaged plant increases.

Despite of the percentage of damaged culms had no overall effect on sediment CH₄ concentration, average concentrations seems to increase until 50% damaged plants treatment and then, decrease towards 100%. A possible explanation for that is when CH₄ release is facilitated by the damage, CH₄ surrounding the roots may will disappear and has to be replenished by the surrounding soil by diffusion or production. When the damaged culms are closer together, the CH₄ may be is not replenished fast enough, which would result in the situation found at 100% damage. It is important to note, that CH₄ oxidation was not directly measured in this study and could have affected our results. Dingemans et al. (2011) evaluated the effect of grazing on CH₄ oxidation in the sediment, but no difference was found between the control and grazed

treatments. We could not find any PMP trend associated with the plant damage levels. However, those samples for CH₄ concentration and PMP were collected near the roots and rhizomes, not actually on them. PMP is a measurement of potential assessed under optimal conditions and therefore, in this particular case, may not have reflected *in situ* conditions. Additionally, herbivory could also affect bubbling CH₄ release (ebullition), since the CH₄ concentration around the roots could decrease faster due the CH₄ escape through the culms, resulting less bubble formation (Dingemans et al. 2011).

In summary, we observed that herbivore damage to *E. equisetoides* can facilitate CH₄ emissions from sediment to the atmosphere, but only under high herbivory pressure. Effects on CH₄ processes (e.g., PMP) that occur in sediment were not evident. Thus, our results, together with those of other wetland studies, further suggest that emergent aquatic macrophytes and their interaction with herbivores may play an important role in regulating CH₄ gas transport within plants, rather than on CH₄ cycling by microbial processes. Despite the experimental approach adopted in the present study, our data may present some limitations. First, we found a very high variability in our CH₄ flux measurements, which directly affects our power to extrapolate our results and support the potential strong effect of herbivory by insects on CH₄ emissions. The chosen time span to sample CH₄ fluxes was relative short compared to other studies (Dingemans et al. 2011; Bhullar et al. 2013), which may have contributed to the observed variability. On the other hand, short time intervals helps to avoid experimental problems such as gas leakage from chambers, ebullition flux from water and the reduction of CH₄ concentration gradient between water and headspace. Second, the levels of herbivory pressure used in the present experiment may be much higher than levels observed in the field. However, our main objective was to test potential effect of the proposed mechanism and our results regarding grasshoppers' impact on

macrophyte stands may only represent a snapshot of actual herbivory pressure, which changes over time and space.

While there are indications that tropical lakes and reservoirs emit much more CH₄ per unit area than similar environments in boreal and temperate biomes (Bastviken et al. 2004; Bastviken et al. 2010; Barros et al. 2011), direct measurements on tropical lakes, where the margins have a crucial role in overall emissions, are still rare. Moreover, we only evaluated a single plant-animal interaction and plant species vary in their capacity to transport CH₄, such variation would influence herbivory-mediated CH₄ fluxes (Laanbroek, 2010). However, Laanbroek (2010) demonstrated that the influence of plant species upon CH₄ emissions is not determined by efficiency of CH₄ transport alone but are affected by the characteristics of site that plants occur, such as sediment composition (e.g, amount of labile carbon). We claim that future research should focus on the contribution of different species to CH₄ emissions, especially focusing on emergent aquatic vegetation and other herbivore-plant interactions, and their influence in CH₄ related processes (e.g. production) as well as the role of abiotic factors in modulating these fluxes.

5. Conclusion

Our results showed that CH₄ fluxes were positively affected by the number of damaged *E. equisetoides*' culms but only under high herbivory pressure (50% and 100% damage). We observed CH₄ fluxes 3.5 times higher in average than undamaged plants mesocosms, thus, our hypothesis about increased percentage of plants with damage from simulated herbivory would be associated with increased CH₄ fluxes was supported. However, CH₄ concentrations and PMP in sediment were not affected. We also performed a field survey in our study system and we revealed that only 4.18% of plant biomass is consumed and this represents, approximately, 15.2%

of damaged culms in *E. equisetoides* stands. This result highlights the fact that herbivorous insects may have a disproportional effect on CH₄ emissions, due to physical damage, compared to their effect on plant biomass loss. In summary, our findings bring a new perspective to the influence of herbivory on the CH₄ and carbon cycling, especially regarding the role that herbivorous insects might play.

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Figure captions

Fig.1. A schematic of the arrangement of the mesocosms in the experimental area and the chamber used for the methane flux measurements. The air in the chamber was mixed by a chamber fan. The chamber was placed over the mesocosm and sealed with a rubber gasket.

Fig.2. Pictures taken in the field (A), showing damage by orthopteran grasshoppers on *E. equisetoides* culms, and taken in the experimental mesocosms (B), showing the simulated insect damage on *E. equisetoides* culms for this experiment.

Fig.3. CH₄ flux (A), CH₄ concentration in sediment (B) and Potential methane production (PMP) (C) measured from mesocosms with different levels of plant damage (0, 20, 50 and 100%). Significant effects of percentage of damaged plants were only observed for CH₄ fluxes.

Figures

Fig. 1

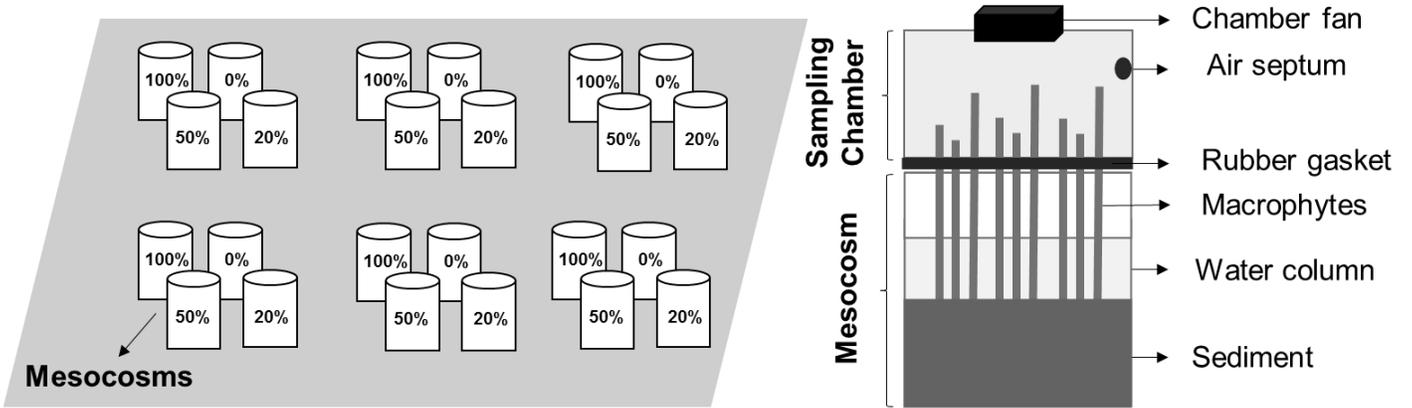


Fig. 2

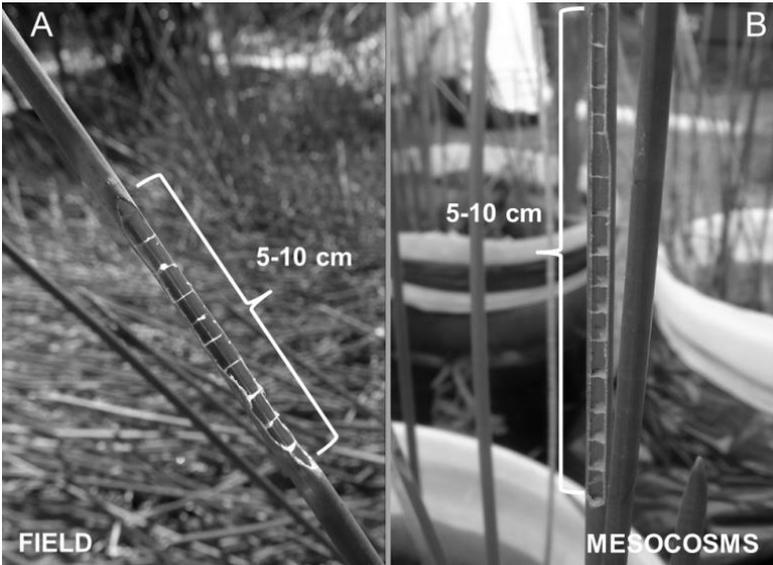
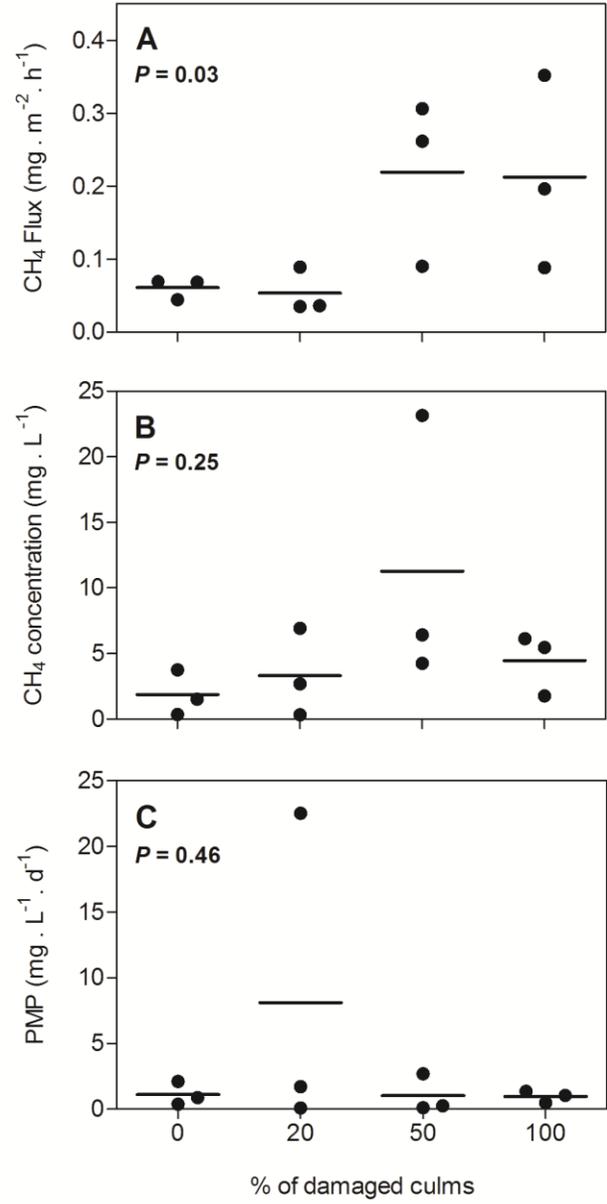


Fig. 3



Supplemental Material

Does herbivore damage increase methane emissions from emergent aquatic macrophytes?

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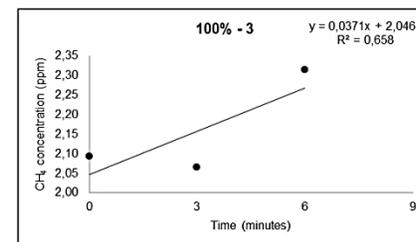
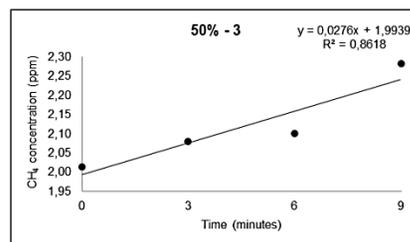
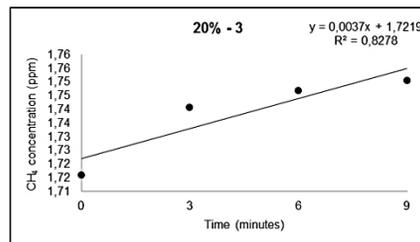
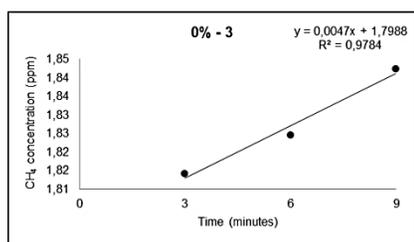
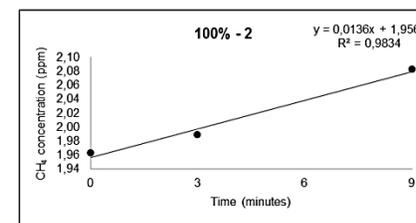
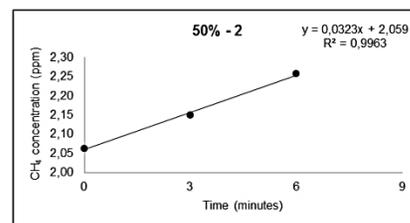
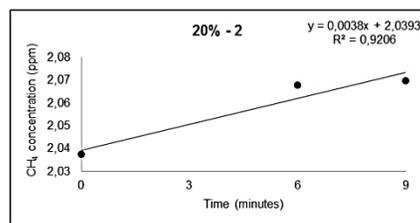
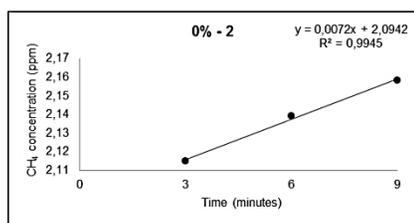
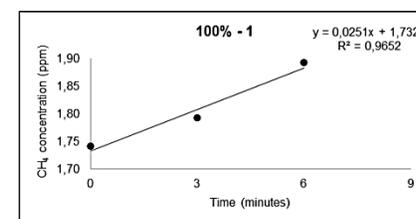
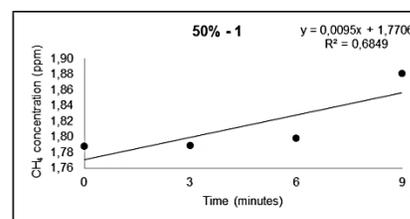
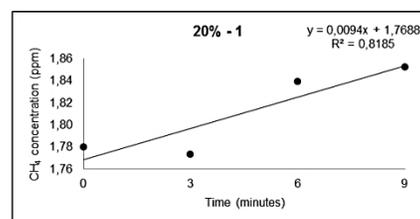
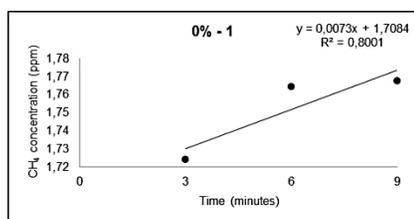
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S1. Linear regressions between methane concentration in the chamber's headspace (ppm) and sampling time (9 minutes) ($n=4$) used for methane fluxes calculations. In the top of each graphic 0, 20, 50 and 100% corresponds to percentage of damaged plants treatments and subsequent numbers are the replicates of each treatment. Only regression equations with R^2 values >0.65 were accepted. For the experiment 50% (twelve of 24) of the mesocosm results were rejected. Some sampling points were excluded because of an erratic jump in methane concentration, probably a result of ebullition during the sampling period.



S2. Linear Regression between dry weight (g) and length (cm) of the *Eleocharis equisetoides* (Elliott.) Torr. culms. The homogeneity of the equation slopes are not significantly different (ANCOVA $P=0.17$).

