QUANTIFYING SEDIMENT METHANOGENESIS AND CARBON DIOXIDE EMISSIONS FROM MACROPHYTE PLANT COMMUNITIES IN A TIDAL HUDSON RIVER WETLAND

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Abstract. Wetlands are the largest natural source of global methane emissions, most of which are generated from methane-producing macrophyte beds. Methane, a potent greenhouse gas, is released from anaerobic methanogenic archaea that break down organic matter. The amount of organic carbon available to methanogens is dependent on the carbon content of the sediments associated with the plant community; therefore, it is important to identify macrophyte populations that have the greatest influence on methanogenesis. Depending on the combination of other factors including dissolved oxygen, dissolved organic carbon, and nitrate in the sediment, wetlands can also be significant sources of CO_2. In this study CH_4 and CO_2 emissions were analyzed from four different macrophyte sediment beds in the tidal Hudson River's Tivoli Bay wetlands. *Vallisneria americana, Typha angustifolia, Trapa natans*, and *Phragmites australis* were studied. Methane emissions ranged from 3.8±4.4 to 11.0±9.7 ppm/112cm^2/min in *P. australis, T. angustifolia,* and *T. natans. V. americana* had the lowest methane emissions at ~0.32ppm/112cm^2/min. Mean CO_2 release from the sediment cores was the greatest from *P. australis* with 15.5±8.3 ppm/112cm^2/min, 10.5±4.5 ppm/112cm^2/min for *T. natans*, 10.5±2.9 ppm/112cm^2/min for *T. angustifolia,* and 1.2±0.9 ppm/112cm^2/min for *V. americana.* The differences in methane emissions were not significant between the four types, but there is sufficient evidence to conclude that if the goal of conservation or construction of artificial wetlands is reducing CH_4 and CO_2 emissions, *V. americana* would be the most ideal species to conserve or plant (conditions willing). Future studies should incorporate other sediment methanogenesis parameters, such as ethanol content and microbial communities, to gain a comprehensive understanding of this complex process. All of these factors need to be kept into consideration as climate change and subsequent changes in hydrology could dramatically alter the current trends in wetland greenhouse gas exchange.

INTRODUCTION

Apart from humans who emit 54-72% of the world's methane (CH_4) gas (Sharifi et al. 2013), it is estimated that wetlands are responsible for 10-40% of the global CH_4 flux, while only making up 4-6% of the earth's land area (Chowdhury and Dick 2013; Grünfeld and Brix 1999; Mitsch and Gosselink 2000; Tian, et al. 2011). With a radiative forcing 25 times greater than that of carbon dioxide (CO_2) (Bridgham et al. 2013; Chowdhury and Dick 2013), CH_4 is a significant contributor to the greenhouse effect. Although a less potent greenhouse gas, wetlands emit greater amounts of CO_2 than CH_4 (Clair et al. 2002). Because of the greenhouse gas effects from CH_4 and CO_2 it is important to identify the major contributors to wetland greenhouse gas production and to quantify emissions.

Depending on the type and density, macrophyte species are significant contributors to CH_4 and CO_2 production, albeit indirectly. Decaying macrophytes supply organic carbon to bacteria and archaea, which in turn, either produce CH_4 or CO_2 during the biodegradation of plant detritus. When terminal electron acceptors used in the ATP production pathway such as oxygen or nitrate are present in the sediment, CO_2 dominates as the byproduct of microbial biodegradation. CH_4, on the other hand, is produced as a fermentative byproduct through a process called methanogenesis (Boon et al. 1995). Methanogens use small carbon molecules as the terminal electron acceptors in the electron transport chain, and the carbon

molecules are converted into CH_4 (Chowdhury and Dick 2013; Sutton-Grier and Megonigal 2011; Tian et al. 2011) Methanogenesis occurs anaerobically so it is ubiquitous in the hydric sediments of wetlands. With 269,252 acres of wetlands in the Hudson River Valley, CH_4 and CO_2 release is predictably substantial (Grigg 2010). In Hudson River wetlands, sediment methanogenesis and CO_2 emission rates were compared between four different macrophyte populations to better understand how aquatic vegetation affects the local greenhouse gas budget.

It is expected that macrophyte populations would differ in CH_4 and CO_2 emissions due to varying sediment characteristics; however, this study focused on the influences of dissolved oxygen (DO), pore-water nitrate, pore-water dissolved organic carbon (DOC), and total organic carbon (TOC) content of the macrophyte sediment beds. When DO is abundant, aerobic decomposition will predominate, preventing the anaerobic methanogenesis process from occurring. CO_2 will then be the dominant byproduct from the aerobic breakdown of organic matter. DO varies spatially as some macrophyte beds may facilitate the presence of oxygen more so than others, leading to differing levels of CH_4 and CO_2 production (Kao-Kniffin et al. 2010). Measuring nitrate was important because it is the most efficient terminal electron acceptor in the anaerobic decomposition pathway of organic matter (Sutton-Grier and Megonigal 2011). If nitrate is present in the sediment, facultative or obligate anaerobic microbes will select nitrate instead of small carbon molecules as the terminal electron acceptors in the fermentation process, inhibiting CH_4 production and leading to CO_2 production.

High DOC in the pore water as well as high TOC in the sediment influences methanogenesis and CO_2 production by providing the fuel source for microbes. Levels of TOC and DOC may differ based on the overall population density of macrophyte species. The DOC in the pore water also depends on the sediment type and how much easily degradable organic matter is present (Jespersen et al. 1998; Miyajima et al. 1997). The amount of readily available DOC is estimated by the decomposability of plant species (Tian et al. 2011). Those that decay the fastest produce the most organic carbon quickly. *T. angustifolia* and *P. australis* have much lower decomposition rates than that of *V. americana* and *T. natans*. T. *angustifolia* has a decomposition rate of about $0.0023d^{-1}$. The decay rate of *P. australis* is $0.0039d^{-1}$ (Chimney and Pietro 2006) *T. natans* is $0.014d^{-1}$ (Findlay, et al. 1990) and *V. americana* has the greatest decay rate of $0.0931d^{-1}$ (Chimney and Pietro 2006). Since *T. angustifolia* has the lowest decomposition rate, it has the greatest amount of lignin and fibrous cellulose/hemicellulose. These structural fibers are much less biodegradable (Miyajima et al. 1997) therefore *T. angustifolia* will most likely produce less DOC than the other species.

Producing the maximum amount of DOC is important, particularly for methanogens, because the terminal electron acceptors ultimately used by the methanogenic archaea are small DOCs leached from macrophyte plant detritus (Boon and Mitchell 1995; Sutton-Grier and Megonigal 2011; Tian et al. 2011). The most readily accessible forms of DOC are simple organic molecules such as methanol, trimethylamine, acetate, and carbon dioxide/hydrogen (Chowdhury and Dick 2013; Tian et al. 2011). However, using organic carbon as the terminal electron acceptor is not the most efficient way of producing energy. In aerobic conditions, oxygen is preferentially used as the terminal electron acceptor by facultative aerobic microbes due to its efficiency as an electron acceptor. When oxygen is depleted the next most efficient electron acceptors are nitrate, sulfate, and ferric iron, respectively (Sutton-Grier and Megonigal 2011). CO_2 and hydrogen are produced as byproducts using these alternate electron acceptors. When the levels of these anaerobic alternate electron acceptors deplete in the sediment, CO_2 and other carbon-based molecules predominate as the terminal electron acceptors, creating an ideal environment for methanogens (Sutton-Grier and Megonigal 2011; Tian et al. 2011).

Which organic carbon molecules are used is dependent on the particular family of archaea. The Methanosaetacae and Methanosarcina families are acetoclastic while the other methanogenic archaea are hydrogenotrophic (Kao-Kniffin et al. 2010; Tian et al. 2011). Independent of the family of methanogen,

the amount of CH_4 generated by methanogenic archaea is correlated to factors including glucose concentration, microbe density, pH (Bergman et al. 1998), plant species presence (Sutton-Grier and Megonigal 2011), and hydrology (Sha et al. 2011; Van der Nat and Middleburg 2000). Sediment depth, temperature, hydrology, and substrate quality are the most influential factors for methanogenesis (Grünfeld and Brix 1999; Wachinger 2000; Tian et al. 2011). Due to the number of non-constant factors that influence methanogenesis, there is great spatial variability in CH_4 generation within wetlands. For this reason, temperature, hydrology, and sediment depth were kept constant in this experiment.

This study involved monitoring methanogenesis and CO_2 emission rates from four different macrophyte beds and correlating the rates to species type and sediment properties. Cores were extracted from each of the four macrophyte beds. Sediment coring was used instead of an in-field gas trap because variability in CH_4 generation within a sediment core is less than that of emissions in the field. This is because the cores isolate microbial methanogenesis and do not account for vegetative CH_4 and CO_2 venting (Van de Nat and Middleburg 2000). Even with cores, although reduced, there is still spatial variability. To address this variability the sediments were characterized for nitrate, dissolved oxygen, dissolved organic carbon, and total organic carbon content; all of which impact CO_2 and CH_4 emissions.

MATERIALS AND METHODS

Quantifying methane emissions from sediment cores

Site and species description

The sampling sites were located in the Hudson River's Tivoli Bays National Estuarine Research Reserve. The bay is divided into a northern and southern bay with a railroad dike running North-South separating the river and the reserve. The *T. natans* bed is found in South Tivoli bay at the mouth of the Sawkill River. South Tivoli Bay is subtidal and is almost always inundated with water (Findlay et al. 1990). The *T. natans* bed is extremely dense and a majority monospecific (Tall et al. 2011). The *V. americana*, *T. angustifolia*, and *P. australis* beds are all within North Tivoli bay. North Tivoli Bay is a predominately intertidal marsh with the most prevalent genera being *Typha*, *Phragmites*, and *Lythrum* (Findlay et al. 1990). The *T. angustifolia* and *P. australis* are both present in the marsh while the *V. americana* is found in the channel of the river west of the railroad.

Each of the four species of interest is found throughout the Hudson River. The most dominant species in the river itself are *T. natans* and *V. americana* while *Typha* and *Phragmites* represent the major cover type in the intertidal wetlands (Nieder et al. 2011)

Sampling

16x4.8cm sediment cores were extracted from each of the four sites. To eliminate any hydrologic variability and to facilitate sampling, cores were taken at low tide. From each site, a total of six cores were collected within a 5 meter radius. Six cores were extracted to ensure an even representation of the spatial variability in sediment organic carbon content and other factors.

Cores were stored in five gallon buckets and water saturation was maintained according to tidal changes. The *V. americana* and *T. natans* cores were stored in one bucket that was completely inundated with water, mimicking the environments they inhabited. Water was changed twice weekly. *P. australis* and *T. angustifolia* cores were stored in one bucket in which the water levels were lowered below the surface of the sediment for three days and then subsequently inundated with water for three days. These cycles were repeated throughout the length of the experiment.

Experimental setup

A gas-collecting water recirculation system was used to collect gas from the sediment cores. A peristaltic pump pulled water from a side-arm Erlenmeyer flask into the top a PVC pipe sediment core. The headspace between the rubber stopper and the sediment of the PVC core ($112cm^2$) was filled with water and completely void of atmosphere. Any CH_4 generated from the core was dissolved into the water and the super-saturated effluent was pumped out of the core back into the side-arm Erlenmeyer flask. The Erlenmeyer flask had 115mL of gaseous headspace filled with inert helium. Every 30-60 minutes, 10mL gas samples were drawn from a stopcock in the mouth of the Erlenmeyer flask and 20mL of helium gas was pumped through the stopcock. Neutral pressure was immediately restored by venting the stopcock. Any water that was discharged into the flask was then pumped back out to the core to again become saturated with CH_4.

Analysis

Gas was sampled from the recirculation system every 30-60 minutes during a six-eight hour time period. The gas was sampled with a syringe and injected directly into a Shimadzu 14-A TCD Gas Chromatograph to quantify CH_4 and carbon dioxide content. To better assess variability in CH_4 and carbon dioxide emission rates from the sediment cores, each of the cores were characterized for dissolved oxygen (DO) content based on depth, dissolved organic carbon (DOC), and total organic carbon (TOC).

Nitrate and dissolved organic carbon were sampled from pore water extracted from homogenized sediment. The top 5.0cm of the sediment was used in the homogenization and the pore water was separated from the sediment using a centrifuge. The supernatant was then tested for nitrate using a SUNA v1.1 and the DOC content was analyzed using a Shimadzu 5050. Finally for TOC analysis 13.0 mL of sediment was massed, desiccated and massed again. Finally the ash-free-dry-mass (AFDM) was taken after combustion in a muffle furnace. The dissolved oxygen content was determined using an oxygen microelectrode.

Data management

Emissions data from some cores were discarded based on one consistently applied rule. After a certain amount of time (around 200 minutes), CH_4 levels began to decrease in concentration within the flask headspace, defying the logical increase in concentration over time. This was attributed to the dilution of CH_4 from the extraction of a sample and reestablishment of neutral pressure with helium. This created an emission rate curve unrepresentative of the actual methanogensis rates. CO_2 was much less sensitive to the dilution due to higher concentrations of CO_2 compared to that of CH_4.

Statistical analysis

After the slopes of the CO_2 and CH_4 emission rates for each core were calculated using a linear regression, normality was determined for each variable using a Shapiro-Wilk Test. *Phragmites* and *Trapa* data from the CH_4 emissions were non-normal while *Typha* data were normal. Due to the non-normal data, a Kruskal-Wallis test was used to compare multiple variables. *Vallisneria* was not included in the Kruskal-Wallis test because its values were so low they were estimated. *Phragmites*, *Trapa*, and *Typha* CH_4 emissions were not statistically different (P-value 0.137).

For the CO_2 data, only the *Vallisneria* data were non-normal. The Kruskal-Wallis test revealed that there was statistical significance between CO_2 rates between the four macrophyte species (P-value 0.000956). Nitrate data were all normally distributed so a single factor ANOVA test was used (P-value of 0.00769). DOC data were also normally distributed so an ANOVA was used (P-value of 0.129). The TOC data from

the *Vallisneria* sediment were non-normal while the others were parametric so a Kruskal-Wallis test was again used (P-value 0.000384).

A linear regression comparing total organic matter content to CO_2 and CH_4 emissions was also used.

RESULTS

Methane and Carbon Dioxide emissions

Mean CH_4 release from sediment cores was 11.0±9.7 ppm/112cm^2/min for *P. australis*, 3.8±4.4 ppm/112cm^2/min for *T. natans*, 6.5±9.2 ppm/112cm^2/min for *T. angustifolia*, and ~0.32ppm/112cm^2/min for *V. americana*. CH_4 emissions from the *V. americana* sediment were below detection limits and no visible peak was provided by the gas chromatograph for estimating peak value. Values were estimated from *V. americana* #2, the only sediment core from the *V. americana* stand that produced any quantifiable levels of CH_4 and should be viewed as the upper bound on CH_4 emission.

Mean CO_2 release from the sediment cores was the greatest from *P. australis* 15.5±8.3 ppm/112cm^2/min, 10.5±4.5 ppm/112cm^2/min for *T. natans*, 10.5±2.9 ppm/112cm^2/min for *T. angustifolia*, and 1.2±0.9 ppm/112cm^2/min for *V. americana.*

Oxygen saturation at depth

O_2 had disappeared by 0.5 – 1 cm in all cores indicating they were all predominantly anoxic with no difference among plant communities.

Sediment TOC content

The *P. australis* sediment cores had the highest average organic matter content of 29.3±5.6%. *T. angustifolia* had 17.8±6.4% organic matter, *T. natans* had 9.1±1.5%, and *V. americana* had the lowest at 4.1±1.4%.

Dissolved organic carbon content and pore water nitrate

The pore water nitrate content of *T. natans* was the highest at 0.99±0.21mg/L, *V. americana* was 0.70±0.33 mg/L, *P. australis* was 0.47±0.07 mg/L, and *T. angustifolia* was 0.33±0.17 mg/L. *V. americana* had a DOC pore water content at 12.18±1.59 ppm, *T. angustifolia* had 10.79±5.77 ppm, *P. australis* had 10.53±1.09 ppm, and *T. natans* had 7.38±0.53 ppm (the DOC values lacked statistically significant differences).

DISCUSSION

Methane emissions

CH_4 emissions from each of the plant bed cores had very high standard deviations and there was significant overlap in the level of methanogenesis between each of the sediment types (excluding that of *V. americana*). This overlap may be attributed to the many variations in sediment conditions. One significant sediment characteristic that would help differentiate CH_4 emissions is the accessibility of organic matter to methanogens (Medvedeff et al. 2015; Sutton-Grier and Megonigal 2011). However, after measuring total organic carbon content in each of the sediment cores and comparing it to CH_4 emissions, there was no correlation between the two. Despite the significant differences in total organic matter content between the sediment types, the widespread spatial variability of other sediment

parameters may have resulted in the overlap between the plant bed CH_4 emissions (Sha et al. 2011). Temperature and water table fluctuations are the principal factors that influence methanogenesis (Grünfeld and Brix 1999; Sha et al. 2011; Van der Nat and Middleburg 2000) but since temperature and hydrology were controlled in this experiment, other sediment characteristics most likely contributed to the variability in CH_4 emissions within each plant community. The other contributors would be ethanol content, variations in microbial communities, pore water organic carbon content, pore water nitrate (acting as the more efficient terminal electron acceptor to small organic carbons), and oxygen saturation in the sediment.

Dissolved oxygen availability would have also contributed to variations in CH_4 emissions but the oxygen content did not differ among sediment types. However, there were differences in nitrate availability between the cores, which may have exhibited a strong control on CH_4 emissions. When nitrate is used in place of organic carbon as the terminal electron acceptor, the microbes produce CO_2 instead of CH_4. The pore water nitrate content of the *P. australis* and *T. angustifolia* were the lowest of the four vegetation types which may have contributed to the higher mean CH_4 emissions. The *V. americana* had the second highest nitrate content at 0.70±0.33 mg/L which may be a contributing factor to the extremely low CH_4 emissions. As for *T. natans* the nitrate content was the highest; however, this may have been balanced out by the coupled high organic carbon content, resulting in overall CH_4 emissions greater than that of *V. americana* but lower than *P. asutralis and T. angustifolia* beds.

Although CH_4 levels could not be precisely estimated from *V. americana*, the sediment cores clearly yielded lower emission rates than the other macrophyte species. This may have been attributed to the relatively high nitrate content or the sediment morphology and location of this particular *V. americana* population. Because the stand was located in the middle of a Hudson River channel, much of the dead organic matter may have been immediately washed downstream instead of permeating the sediment for microbial use. Another explanation for the reduced *V. americana* CH_4 emissions is their root depth. Deeper roots undergo greater fermentation, thus expelling greater amounts of ethanol. When ethanol is present, it is bio-transformed into acetate. Since a majority of methanogens are acetoclastic, the release of CH_4 may be increased with more complex and deep root structures (Williams and Yavitt 2010). Roots of the other three species were deeper thus possibly contributing to greater acetate formation.

Carbon dioxide emissions

Unlike CH_4, CO_2 emissions did show a positive correlation with increasing TOC levels, but that is because there is sufficient reason to believe that CO_2 emissions are less sensitive to spatial variability in sediment characteristics than that of CH_4. *P. australis* emitted the greatest amount of CO_2 followed by *T. natans*, *T. angustifolia,* and *V. americana.* From this and other experiments it is reasonable to conclude that CO_2 emissions are greater than that of CH_4 from wetlands (Clair et al. 2002).

Further considerations

One reason why CH_4 production is limited and CO_2 is more prominent is that some CH_4 is consumed by methanotrophic archaea and bacteria (Chowdhury and Dick 2013 and Kao-Kniffin et al. 2010). Methanotrophy is present in the uppermost layers of wetland sediment and in the margins between sediment and water (Chowdhury and Dick 2013). The methanotrophs consume CH_4 and produce carbon dioxide as a byproduct. It is estimated that the aerobic oxidation of CH_4 mitigates 40-70% of global CH_4 emissions (Bridgham, et al. 2013) but this mainly accounts for terrestrial methanotrophic microbes (Chowdhury and Dick 2013). There is little to no research on the influence of wetlands as CH_4 sinks, mostly because wetland sediments are predominately anaerobic. Despite the aneaerboic conditions, Chowdhury and Dick suspect that oxidation of CH_4 by methanotrophs is still significant but only vastly understudied (2013).

Another important consideration concerning methanogenesis is that CH_4 may also be transported through macrophyte tissue and vented out the leaves (Kao-Kniffin et al. 2010; Van der Nat and Middleburg 2000). Carbon dioxide is also vented out the leaves through plant respiration (Clair et al. 2002). In vegetated sediments, this form of plant-mediated transport, as opposed to ebullition, accounts for 62% of the CH_4 emissions (Grünfeld and Brix 1999). Despite the prominence of this pathway, only direct release of the two greenhouse gases from microbes to the water and atmosphere were accounted for in this study as live plants were not present in the cores.

Despite the variability and lack of significant difference in CH_4 emissions from each of the sediment types, it is still reasonable to conclude that certain macrophyte beds contribute to greater CH_4 emissions. For example, *P. australis* and *T. angustifolia* sediments could emit up to 50% and 30% respectively more CH_4 than *T. natans*. If recommendations were to be made for implantation of macrophyte species in artificial wetlands or to selectively preserve certain macrophyte species to minimize CH_4 emissions, it is reasonable to choose *V. americana* and *T. natans* as ideal species. They emit substantially less CH_4 than that of *P. australis* and *T. angustifolia*. As for CO_2 emissions, *V. americana* beds emit a significantly lower amount of CO_2 than any of the other species that were examined.

CONCLUSION

Monitoring CH_4 flux from wetlands is an important but a difficult subject to study. Due to the influence that wetlands have on the global greenhouse gas budget, understanding to what degree certain wetland characteristics contribute to the greenhouse gas flux is imperative. Yet the spatial variability in wetlands leading to differential CH_4 emissions, even within a single plant population, makes studying greenhouse gas emissions challenging. There could be differences in sediment quality (amount of organic matter present, TOC and DOC), oxidation by methanotrophs, availability of alternative terminal electron acceptors, ethanol content, temperature, hydrology, pH, salinity, and densities and composition of microbe populations. Future studies should consider each and all of these factors when comparing sediment type to CH_4 emissions. Furthermore, this study only focused on the ebullition of CH_4 from sediments because it was conducted in a laboratory setting. Although there may be even greater environmental spatial variability because temperature, hydrology, and sediment depth are not controlled, gas traps may provide a better idea of CH_4 emissions from wetlands. This is in part due to the fact that up to 62% of CH_4 emissions from wetlands are vented through the vascular tissue of plants instead of bubbling from the sediment itself (Grünfeld and Brix 1999).

Although it is possible that carbon sequestration may outweigh methanogenesis in wetlands (Clair et al. 2002 and Kao-Kniffin et al. 2010), carbon sequestration and methanotrophy may not fully alleviate the potential long-term consequences of changing wetland greenhouse gas dynamics. Wetland CH_4 and CO_2 production remains an important process to study and should be of continued interest to the scientific community due to the impending threats of climate change. Climate change could exacerbate wetland greenhouse gas emissions, creating a positive feedback loop. The presence of salt from sea level rise, hydrologic changes, alterations in macrophyte species type, and temperature changes could potentially amplify the present trends of wetland greenhouse gas release (Clair et al. 2002; Grünfeld and Brix 1999; Medvedeff et al. 2015; Sutton-Grier and Megonigal 2011). These factors could also alter the efficacy of wetlands to act as carbon sinks and upset the balances between methanotrophy and methanogenesis (Stefanik and Mitsch 2014). To better understand and protect the environment, continued research on these important and dynamic ecosystems is imperative.

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APPENDIX

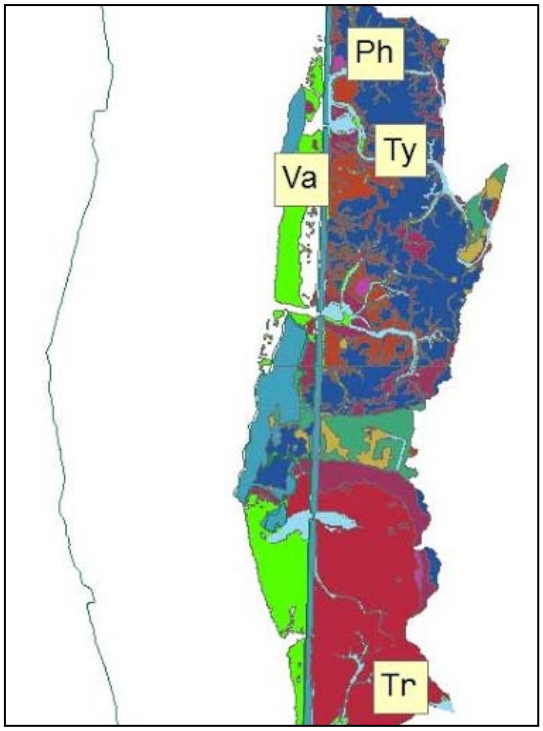

FIGURE 1. Map of macrophyte coverage and sampling sites in the Tivoli Bays National Estuarine Reserve. The coordinates for the sampling beds are as follows:
Trapa: 589127,4651773
Typha: 589338,4655269
Phragmites: 589252,4655876
Vallisneria: 588855,4655173

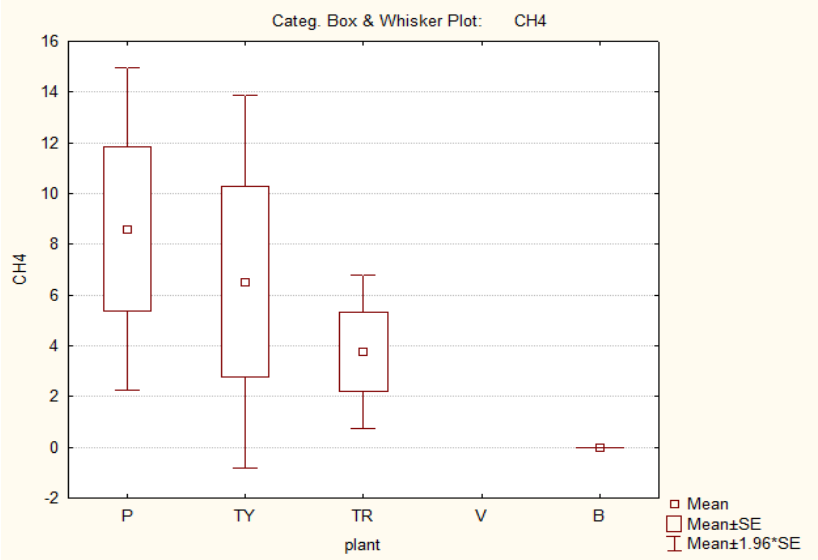

FIGURE 2. CH_4 emissions from each of the sediment types.

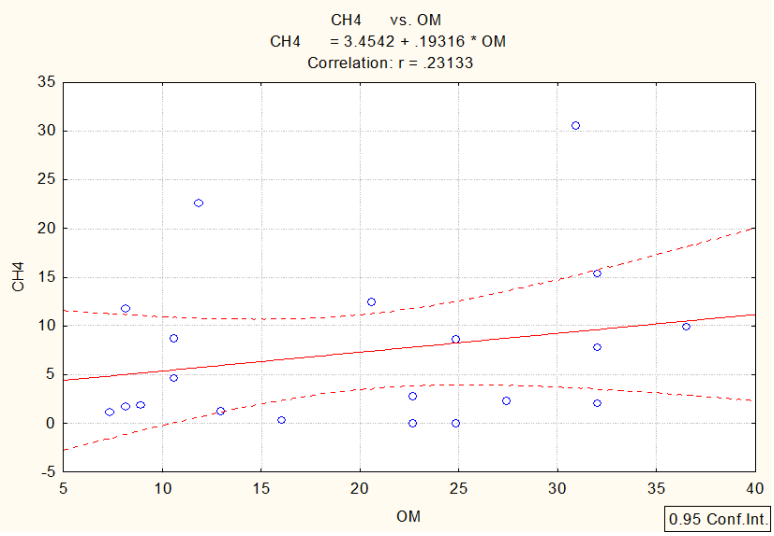

FIGURE 3. Comparing CH_4 emissions to organic matter content amongst cores.

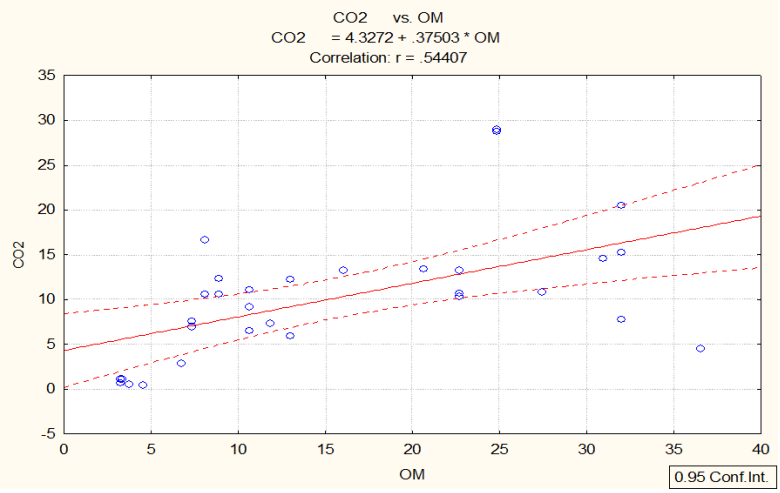

FIGURE 4. Comparing CO_2 emissions to organic matter content amongst cores.