Artificial Wet Buffer Zone: Sink or Carbon Source/Balance sheet approach

Anandita Agarwal

ÉCOLE POLYTECHNIQUE

MSc&T Eco-technologies for Sustainability and Environment Management

Academic Year 2018-19

Agarwal Anandita

INTERNSHIP REPORT

« Artificial Wet Buffer Zone: Sink or Carbon Source/ Balance sheet approach »

Referent teacher: Stephane Bouchonnet
Internship tutor: Julien Tournebize; Cedric Chaumont
Internship dates: 25 March – 31July 2019

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Abstract

Wetland provides important ecosystem services and store carbon dioxide but is also an important global source of GHG mainly carbon dioxide and methane- a potent greenhouse gas. In order to understand the dynamics of carbon budget for our study area; Rampillon buffer zone is an artificially constructed wetland designed to capture the titled agricultural drainage located near Nangis in Seine-et-Marne in order to improve the surface water quality. Quantifying and understanding the environmental drivers of carbon sequestration from the wetland is important in order to have a better understanding of the current and the future GHG budget of aquatic systems and the global ecosystem as a whole. In this study, we present the observation of carbon storage in sediment coming from agricultural drainage for the year 2017-2018, carbon captured in the vegetation during the growing season of the year 2018, observation of total inorganic and total organic carbon dissolved in the water by continuous measurement via SCAN spectrophotometer since year 2014-2018, observation of CO_2 fluxes by Eddy Covariance tower installed at the site during the one full year or one full growing season of 2017 and measurement of methane fluxes by Automatic Chamber method done via four campaigns held from May 2014 to October 2015. We found that, wetland acts as higher carbon sink via sediment than the vegetation with 2.3±0.248t C- CO_2 of Total Carbon added each year by sediment compared to only 11.5kg C or 42.24kg CO_2 eq via vegetation. Using Eddy Covariance tower to measure CO_2 fluxes, we determined that the wetland is net carbon source with ~1005kg C- CO_2 been released into the atmosphere annually; primarily correlated to ecosystem respiration during the winter indicating the role of soil temperature, microbial activity and atmospheric pressure. Methane fluxes were measured by automatic chamber during the four campaigns resulted in wetland being source of CH_4 releasing 40.85kg CH_4-C or 1021 kg CO_2eq per year; we found distinct diurnal and seasonal pattern of flux rates with ebullition acting as the dominant transport pathways contributing 89% of total emissions. We conclude that the temporal dynamics of methane emissions over the seasonal and diurnal times scales should not be ignored. By studying all the compartments of an artificial wetland and calculating Net carbon budget, we can conclude that Rampillon since its construction i.e. in last 8 years, is a carbon sink with 2.3t C- CO_2 or 8.4t CO_2eq net carbon being stored in the wetland every year with sediment being foremost dominant compartment whereas from Net gas budget; a carbon source with 12.6t CO_2eq of carbon dioxide and 1.02t CO_2eq of methane gas fluxes emitted every year. Further studies are required to investigate many other factors in depth such as soil temperature, atmospheric pressure, relative humidity, microbial activity, photosynthetically active radiation along with reducing the percentage of uncertainties of each compartment to have much deeper understanding of the GHG contribution.

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1. Introduction

Wetlands offer many ecosystem services to humankind including water quality improvement, flood mitigation, coastal protection, and wildlife protection (Mitra, Wassmann, and Vlek 2005; Bohemen 2008). It is estimated that 20–30 % of the Earth's soil pool of 2,500 Pg of carbon (Lal 2008) is stored in wetlands (Roulet 2000; Bridgham et al. 2006), although wetlands comprise only about 5–8 % of the terrestrial land surface (Bohemen 2008). Because of their anoxic wet conditions, wetlands are optimum natural environments for sequestering and storing carbon from the atmosphere. It is also estimated that wetlands emit 20–25 % of current global methane emissions, or about 115–227 Tg-CH_4 year-1 (Whalen 2005); Bergamaschi et al. 2007; Bloom et al. 2010).

While wetlands are productive environments and serve as a sink for CO_2 (Bernal and Mitsch 2013; Bohemen 2008; Waletzko and Mitsch 2013), the carbon sequestration potential of wetlands may be annulled in the short term (on the order of decades) by their methane (CH_4) emissions (Bernal and Mitsch 2013). Methane is a green-house gas roughly 25 times more potent than CO_2 when considered over a 100-year horizon (Stocker et al., 2013) and is widely associated with wetlands.

Publications that emphasize the comparison of the two major carbon fluxes in wetlands are relatively few. (Mitsch et al. 2013 and Mitsch and Gosselink (2015) illustrated by dynamic modeling of carbon flux that methane emissions become unimportant within 300 years compared to carbon sequestration in wetlands. Within that time frame, most of the wetlands became both net carbon and radiative sinks. The only wetlands that remained net radiative sources in these comparisons were peatlands that were already sources of CO_2 caused by drainage.

The standard global warming potential (GWPM) used by the international panel on climate change (Solomon 2007) and others to compare methane and carbon dioxide is now 25:1 over 100 years. This GWP ratio is used by policy makers to compare methane and carbon dioxide fluxes. (J. WHITING and P. CHANTON 2003, Fuglesvedt et al. 2003) and (Xiao et al. 2006) all expressed concern about using a constant methane GWP factor because: (1) a longer period (100–500 years) should be considered for sustainable ecosystems such as wetlands (necessitating a dynamic modeling approach); and (2) since GWPs are constructed to express equivalence in terms of the radiative forcing over a chosen time horizon of pulse emissions of different gases, the GWP does not consider persistent sources and sinks well.

Methane's lifetime in the atmosphere has been reported as 8-10 years before being oxidized to CO_2 (Fuglesvedt et al. 2003; Schmidt 2004) but using a 7 year half-life and a first order decay constant in the two-state-variable model reported in the literature. Using this model for seven wetland, each has a ratio of carbon dioxide decrease to methane increase well above the GWP_M of 25:1 within 300 years (Mitsch et al. 2013). The natural temperate wetland, with the ratio of 71:1 after 100 years, becomes a sink after 31 simulated years. For created temperate zone wetland flow through slough, 255 kg of CO_2 are taken out of the atmosphere for every kg of CH_4 increase in the atmosphere after 100 years. Previously published methane emission rates measured in the tropics/subtropics include 12–22 g-C / m^2/ year in Australian billabongs (Sorrell and Boon 1992), 3–225 g-C / m^2/year in Louisiana freshwater marshes (DeLaune and Pezeshki 2003), 30 g-C / m^2/year in the Amazon Basin. (Mitsch et al. 2013) showed created wetlands had methane emissions lower than or comparable to natural wetlands after 13-15 years.

The definition defines wetlands as “land, whether logged or uncultivated, usually flooded or gorged with fresh, salt or brackish water, either permanently or temporarily; vegetation, when it exits, is dominated by hygrophilous plants for at least of the year. It is supplemented by a set of criteria specified in the decree of 24 June 2008, which recognizes the anthropogenic origin of an area provided that it satisfies a minimum of ecological characteristics (Cizel, 2010). It is in context that a project to create an artificial wet buffer zones was launched in Rampillon, before materializing from 2009 and being rigorously monitored by IRSTEA. According to the technical guide to the implementation of the artificial wet buffer zones carried out by IRSTEA in 2015 (Tournebize et al. 2015), a ZTHA (Zone Tampon Humide Artificielle) in an agricultural environment is a retention basin, a buffer interference between agricultural land and river, to monitor water quality issue.

The degradation of freshwater quality is a growing problem that disrupts ecosystem and leads to reconsider the relevance of alternative purification systems (Turpin et al., 1997). This stake is illustrated in the case of the protection of groundwater resources of the Champigny water table in Seine-et-Marne (77) which supplies drinking water to nearly one million people in Paris region. Its recharging system is specific since 70% of it is carried out through direct surface water / groundwater exchanges within an agricultural context of arable crops. The territory of Seine-et-Marne is characterized by an intensive cereal production and a surface 100% drained around Rampillon which involved the transfer of fertilizers and pesticides products. This agricultural intensification has a significant impact on the ecosystem of the district, particularly through the diffuse pollution causing an imbalance of nitrogen and pesticides in the water.
Carbon cycle and artificial wet buffer zone are closely related elements. What is called carbon sequestration involves a set of chemical transformations within the cycle leading to the return of the element in its gaseous form, its extraction from the physical medium and its release in to the atmosphere (Kadlec 2005,Mitsch et al. 2005). Precise quantification of fluxes in terms of mass and concentration of different biological forms of carbon remains complex and uncertain. A set of physical, chemical and biological parameters influence the cycle. The internship explores the relationship of wetland – carbon cycle and studies the different pathways of dissipation of carbon.

Organic carbon inputs to wetland systems originate from both exogenous (terrestrial plant debris and eroded soil material) and endogenous (plankton and aquatic macrophytes) sources. Constructed wetlands receiving agricultural runoff have the potential to sequester exogenous and endogenous carbon through processes such as sedimentation and in-situ primary production (Maynard, Dahlgren, and O’Geen 2011).

Atmospheric carbon (CO_2) is sequestered in the pedosphere through the production of organic carbon (OC) by photosynthesis (Stryer, 1995). Oxidation of OC through natural and agricultural processes (Amiotte Suchet, Probst, and Ludwig 2003) liberates CO_2 from organic compounds, and this carbon subsequently returns to the atmosphere or reacts with carbonate rocks (chemical weathering). The presence of Ca2+ ions in the soil profile is beneficial for crop production because they can buffer pH changes related to chemical perturbations and provide beneficial cation exchange capacity to the soil (Barak et al. 1997).

Carbon input provides the fuel for the denitrification process in the soil, converting nitrates to nitrogen N2. C/N (Kg) ratio found in our study as to be 17.4 which serve to understanding the amount of liable carbon and nitrogen present for decomposing as well as denitrification.

However, still much is unknown about the effects of wetland management on carbon fluxes (Zedler and Kercher 2005). In the last two decades the eddy covariance (EC) method has been widely used to measure net ecosystem (NEE) of CO_2 in wetlands, mostly in peatlands ((Neumann et al. 1994); (Aurela, Tuovinen, and Laurila 1998); (Joiner et al. 1999); (Arneth et al. 2002; Lafleur et al. 2003; Corradi et al. 2005; Glenn et al. 2006) but also in marshes (Bonneville et al. 2008; Schafer et al., 2014).

There is significant gap in the quantity and control of carbon sequestration and methane CH_4 release of wetlands (Zedler and Kercher 2005) especially in rural areas. Current estimates of carbon uptake strengths of temperate wetlands have an uncertainty of more than 100% (Bridgham et al. 2006). Chamber accumulation measurements are widely used, but due to their labor intensive nature are, sampled intermittently. The chamber approach has several sources of potential biases which may impact its ability to be scaled up to ecosystem level estimates (Sachs et al. 2010). One such potential source of bias is driven by the non-random temporal sampling intermittency of chamber measurements, as samples are typically concentrated during the day and the growing season, and the assumption that flux rates are negligible in other times and uniform during the period represented by the sample. Recent studies using the eddy covariance technique to measure CH_4 emissions report a large variations in flux rates between specific microsites within the tight mosaic of patches that wetlands are composed of (Baldocchi et al. 2012; Forbrich et al. 2011; Sachs et al. 2010). The infrared gas analyzer technology for high frequency CH_4 concentration (Kormann, Müller, and Werle 2001) can be used with EC technique to provide continuous measurement of CH4 fluxes from the integrated footprint area that is representative of a large region of the wetlands.

Methane generations occurs in anoxic wetlands soils and is dependent on the temperature, the level of anoxia, the availability of the liable carbonous substrate, and the reduction potential of the soils (Sha et al. 2011; Updegraff et al. 1995). Methane must then be transported to the surface. Transport can occur in one of the three ways: (1) Diffusion, by following a concentration gradient of methane between the soil, water and the atmosphere. Diffusion is a relatively slow process and diffusion rates will vary slowly in time. (2) Plant mediated i.e. aerenchyma, by escaping through porous plant tissue. This is pathways for methane emission is expected to be correlated with the plants' stomatal conductance that governs the transport rate from intercellular spaces to the air. (3) Ebullition, or bubbling through the soil and water. This process occurs when methane beyond the saturation limit of the aqueous phase may form into bubbles and spontaneously rise to the surface of the wetlands, transporting potentially large amounts of methane in a very brief period of time. The intermittency of ebullition makes it more difficult to model accurately and can have a profound influence on the observed methane flux levels. These different transport mechanisms needs to be understood to find correlation between the methane fluxes from different medium. Some recent models incorporate a combination of diffusion, ebullition and aerenchymal transport of methane to the atmosphere (Riley et al. 2011; Walter and Heimann 2000; Wania, Ross, and Prentice 2010), though some do not yet include convective gas flow as a mechanism for transport. Ebullition is found to be the major contributor of methane flux in our study, with little diffusion phenomenon while stomatal conductance have not been investigated in this study.

In most EC measurements over the wetlands, it is challenging to fulfill all the theoretical requirements of eddy covariance or follow the guidelines provided by the eddy flux community (Aubinet, Vesala, and Papale 2012); (Baldocchi et al. 2012) due to heterogeneous nature of wetlands and the fetch limitations. Previous studies have used a flux footprint model to interpret how spatial heterogeneity contributes to the variability in EC fluxes in the wetland (Forbrich et al. 2011; Sachs et al. 2010; Matthes et al. 2014). Since the source area that contributes to the eddy covariance flux changes with the wind direction, EC

measurements combined with the footprint modelling can be used to partition NEE dynamics of vegetation covers within the tower footprint area. The same technique has been utilized in our study.

The emissions of carbon dioxide CO_2 and/or methane CH_4 are driven by local soil conditions, however the responses vary widely. As the oxygen is depleted upon abrupt wetting or over an extended saturation period, soil respiration is generally predicted to proceed by sequential consumption of nitrate, manganese, iron, sulfate and ultimately, methanogenesis. As a shift in terminal electron acceptors and products of microbial metabolism are integral to determining the temporal dynamics of measured process, they will in turn determine the dominant C loss mechanism from a soil system. Recent studies ascribe soil C efflux responses to moisture perturbations to "soil moisture legacy effects" (Evans and Wallenstein 2012; Banerjee et al. 2016) contingent on the extremity and duration of the perturbation. (Morin et al. 2014) shows there is a seasonal variation and diurnal pattern observed in the CH_4 fluxes.

The motivation of this study is therefore to better understand the effect of non-point source pollution and mitigation effects of constructed artificial wetland in determining the carbon mass balance from the wetland. In order to do so we will be focusing mainly studies on Water [Total Organic Carbon TOC, Total Inorganic Carbon TIC], Gases [Carbon-Dioxide CO_2, Methane CH_4] and Carbon in Soil, Sediments and Vegetation from the wetland.

2. Materials and Methods

2.1 Presentation of the Study Area

Experimental Site: Rampillon

The experimental site is representative of the infiltration of drainage water through sinkholes down into the Champigny aquifer. The catchment is located in the city of Rampillon (0303'37.300 E, 4832'16.700 N, 70 km south-east of Paris, France) and the total drained area is 355 ha (according to subsurface drainage network maps). The average annual air temperature is 10.5 C, the annual mean rainfall is 689 mm and the annual mean potential evaporation is 679 mm. Most of the basin, covered with tableland loess up to 10 m thick, is relatively flat and sub-horizontal. The soil of the catchment is mainly Luvisol (FAO, 1998). The unconsolidated silty deposits, about 2 m thick, have developed a textural clay layer surmounting silex clay or loamy clay (100–140 cm, and more than 140 cm, respectively, on average). All the clay layers behave as an impervious layer and allow a temporary perched water table that can reach the soil surface in wet periods. Consequently, two water tables may coexist: one in the limestone Brie Formation aquifer and the other in the perched water table. The temporary perched water table is subsurface drained by pipes buried at a depth of 90 cm and spaced 12 m apart. The connection with the limestone occurs when the clay layers disappear or are pierced by sinkholes. Originally conceptualized to represent 1.5% of the surface of the sub-watershed, the site has a surface area of 5,300 m^2 for a volume of 2,500 m^3, that is to say 0.15% of the catchment area. This corresponds to a useful volume of about 7 m^3 per hectare drained. The ZTHA has three communicating basins, through which the ditch creek water passes. The upstream basin has a volume of 300 m^3, and serves as a sedimentation unit where the energy of the input flow is dissipated. The intermediate zone has an initial capacity of 1000m^3 and recreates a relatively rugged micro-topography with the aim of diversifying the habitat of the zone. It is built with the goal of maximizing both biodiversity and purification potential. Finally, the downstream basin has a dead volume of 500 m^3 and useful volume of 1000 m^3 which leads to the outlet

value of the ZTHA. This rejects the water in the collector ditch that runs along the wetland and empties into the chasms.

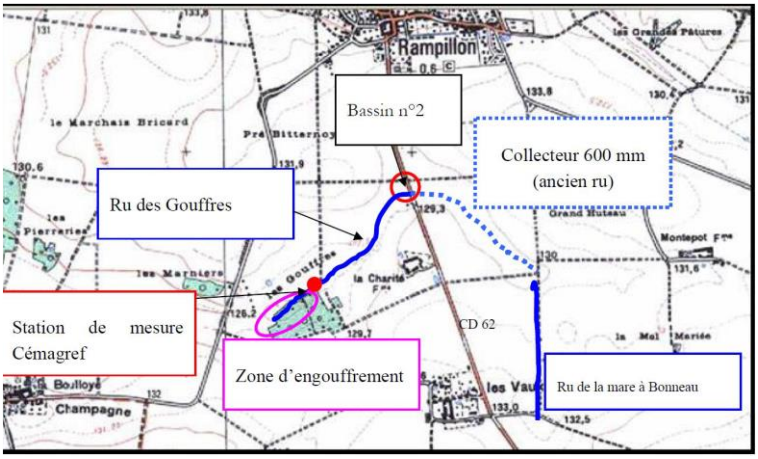

Figure 1 : Presentation of the hydrographic network of the Ru des Gouffres basin. Aquibrie-2008.

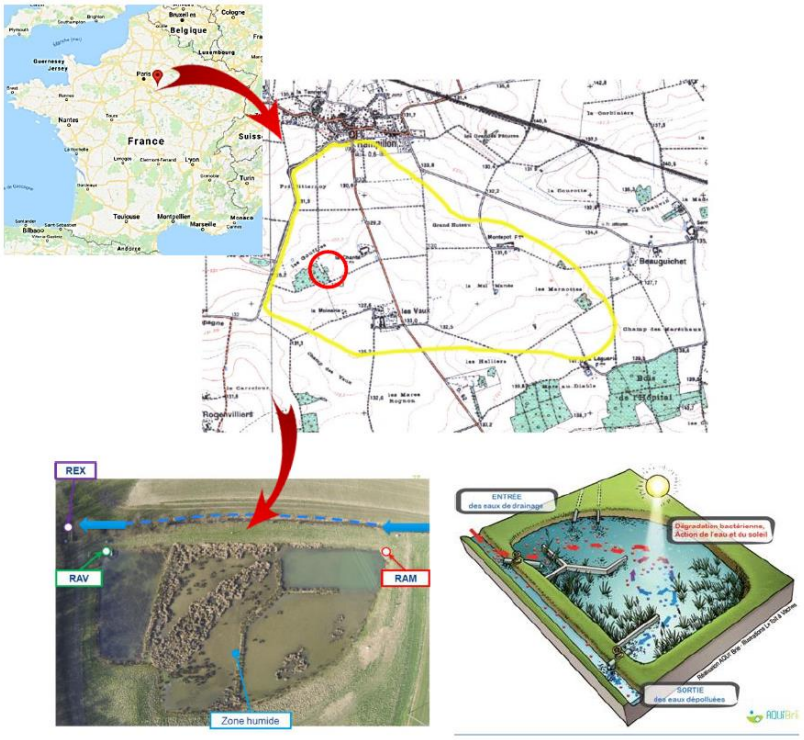

Figure 2 : Diagram of location and visualization of the Rampillon wet buffer zone, design and operation. Adapted from Tournebize 2014, internal documents of the IRSTEA team, Google Maps.

2.2 Hydrology and Plant Stand:

The area is currently planted at 39% (May 2018) following an episode of massive disappearance of *Carex, Typha* and *Phragmites*. Originally, however, all three basins were artificially vegetated by stands of *Carex* and islands of *Phragmites* beds in the periphery (*Phragmites and Typha,* local species) approx. ~ 14%. The slopes and immediate surroundings of the basin have been planted with a seed mixture of grasses and other species adapted to the wetland (Aqui'Brie, 2008a). The work of Manon Louis (trainee

IRSTEA in 2012) showed a change in the plant population two years after the creation of the area through significant changes in the dominant species in the area. Thus, the Rampillon artificial wetland had a high density of *Carex riparia* approx. ~ 13% in 2012 in place of *Carex acutiformis*, a weak colonization by the initial species *Juncus effusus*, the appearance of fast-developing species of *Juncus* type (mainly *inflexus*) in the intermediate basin approx. ~ 9%, and the complete disappearance of the *Typha Latifolia* plants following their consumption by nutria (*Myocastor coypus*).

The volume of water contained in the artificial wetland varies from one season to another and depends heavily on rainfall and hydrological regime. As a reminder, the basin is fed mainly by drainage water agricultural parcels Ru d'Ancoeur watershed. The amount of water discharged by the drains depends on the rate of soil water saturation, which generates a drainage flow when it exceeds the holding capacity. The water discharge interception for the year 2014-2016 was approx. ~ 50% of annual diurnal flow (150 mm/yr.). Based on the observed heights and the 8 years of existence of the zone, the rate of accumulation of sediments does not appear homogenous from one zone to another. It is on average 2.5 cm/ year with values ranging from 4.4 cm/ yr. in zone 1 to 1.25 cm / yr. in zone 3. This heterogeneity is not surprising since the tracing operation conducted at Rampillon (29/03/2017) showed that the movements of water within the basin are not characterized by the same flows. The speed in zone 1 is lower which allows more time for sediment to accumulate.

2.3 Data Analysis & Methodology:

2.3.1 Carbon Quantification in Sediments

The estimation of the carbon contained in the sediments of the wetlands passes through several stages: sample collection, Laboratory analysis of particulate inorganic carbon and organic carbon, estimate of the total sediment volume contained in the area as of March 2018 (sampling date). The protocol is established using the continental sediment sampling and pre-treatment guide for physio-chemical analyzes of the water framework directives (M. Coquery April 2010). Due to the configuration of the wetland, the depth and the accessibility of the different basins, as well as the work of previous trainees, five sampling points are determined and associated with five geographical area (Figure 3).

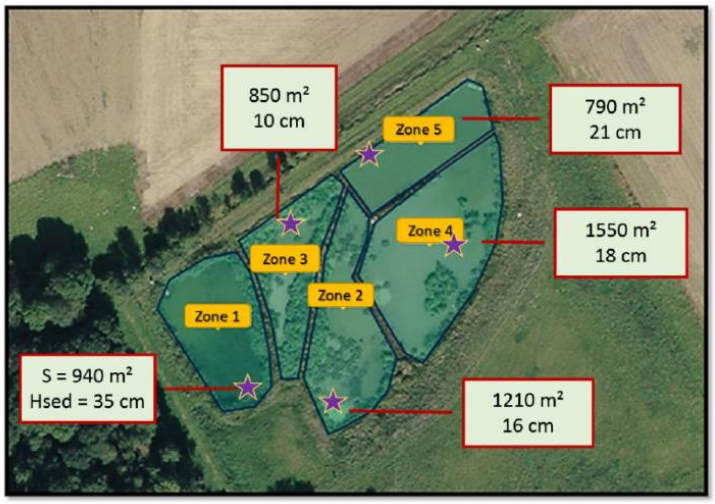

Figure 3: Sediment sampling points and the sediment height from each zone. S = surface area of each zone, Hsed = Sediment Height of t=each zone

The protocol is twofold: two collect the samples for laboratory analyzes and in order to estimate a total volume of sediments (Appendix 1). At each sampling point, three samples are recovered in the first 15 cm of soil by removing as much debris (soil and animal debris) and the layer of organic surface material. These samples are then mixed and homogenized before analysis. In order to estimate one sediment volume per zone, two metal cylinders with volume of 251 cm^3 (height of the cylinder 5cm, diameter of the cylinder 8cm) are filled with sediment by sampling point. At the same time, water height and sediment height are measured. This method associates a weight of dry sediments with a given volume, allowing extrapolation of total mass of sediments to the entire wetland (Figure 3). As we know the surface area of each zone along with the height of the sediment, we can calculate the volume of sediment per zone. Similarly by knowing the weight of the sediment and volume of the measuring cylinder, we can calculate density of sediments per zone. As we know the volume and density of the sediment, total carbon particulate as well as organic carbon is calculated ranging from 2.88 t in zone 3 to 9.6t in zone 4 and 0.46t in zone 5 to 1t in zone 4 respectively .The sediments are kept in a cold room before being homogenized and weighted wet. For the estimation of the values of organic carbon, sediment is decarbonized by acidification to remove all the inorganic carbon. The measured IC consists of carbon derived from carbonates converting calcium carbonate to carbon dioxide. Further, they are dried in an oven at 105° C and then ground. The results of carbon are provided by elemental analyzer. For the estimation of the organic carbon, the protocol differs and is provided in Appendix 1.

2.3.2 Carbon Quantification of Plant species:

As in the case of sediments, the estimation of carbon contained in the plants of the wetlands passes through stages: The updating of the inventory of the species present on the sites, as their spatial location (Figure 4), sample collection within the wetland, laboratory analysis of the organic and inorganic carbon. Samples are taken in mid may during the growth phase of the majority of the species, as this is the optimal period to evaluate the absorption of carbon by plants. The majority species present on the site are common Reed (*Phragmites Australis*), the Rush (*Juncus Inflexus*), the Sedge (*Carex Riparia*) and the hydrophyte submerged Callitriche (*Callitriche Palustris*).

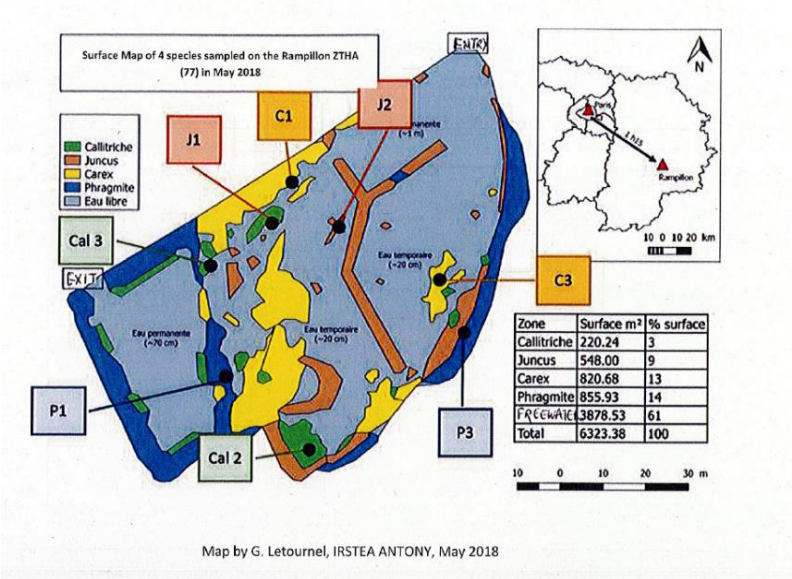

Figure 4: map of the distribution of the four species sampled on the Rampillon area with location sampling points. Created on QGIS by G. Letournel and M.Blandin from a drone image of 23/05/2018 taken by IRSTEA.

The reference document used to establish the field and laboratory protocols was developed by IRSTEA in 2015. This is the detailed protocol for harvesting plant biomass in vegetated areas of release, dealing with the role of the plant compartment within the plant compartment. The plants are distinguished according to the classification of Brix (1989) which is chosen as the basis of differentiation of macrophytes. In this context, the species to be sampled are all emerging macrophytes with stem and aerial leaves but with a root system on submerged substrate, with the exception of Callitriche which is classified as a totally submerged macrophytes. The protocol differs according to these categories and is detailed in the Appendix 2. It should be noted that a pre-release of the field made it possible to partially test the protocol and to adjust it following the observation of certain practical difficulties. These are also specified in the Appendix 2. The analysis of carbon compounds in the living matter requires a careful preparation work, a crucial element of which is the cleaning and drying of plants. Similarly, the complete protocol is detailed in Appendix 2.

2.3.3 Carbon Quantification in Water: TIC/TOC data:

TIC/TOC is measured by the three SCAN probe installed on site; first two being installed at the inlet and the outlet in the wetland and third being used in the laboratory for water sampling measurement, and as well as by the point water sampling method. SCAN probe (Immersible compact spectrometric analysis probe) which continuously measures the concentrations of nitrate and organic carbon total. An automatic sampler installed at the site also makes it possible to collect samples at fixed frequency according to variations in the flow rate. Samples were taken and manual measurements in the laboratory are also performed. The total dissolved organic carbon is measured in the stream.

TOC/DOC measured in LAB is done via hot oxidation method by extractive gas analyzer. This method consists in oxidizing all the organic matter into CO_2 by high temperature heat treatment. This CO_2 is then measured by non-dispersive sensor probe NDIR (Appendix 3). Third SCAN probe installed in the LAB, is based on a measurement of UV/Visible absorption spectroscopy. It is based on the absorption capacity of the UV light by the organic matter. The entire absorption spectrum of the solution over a wide range of wavelengths is measured. This spectrum reflects in a complex way the presence of nitrates, nitrites, anionic surfactants, organic matter dissolved colloidal and solid particles in suspension. Using reference spectra for each of these classes of constituents, the signal is de-convolved by an algorithm that allows the calculation of the most probable concentration of each of these constituents (Thomas et al., 2007).

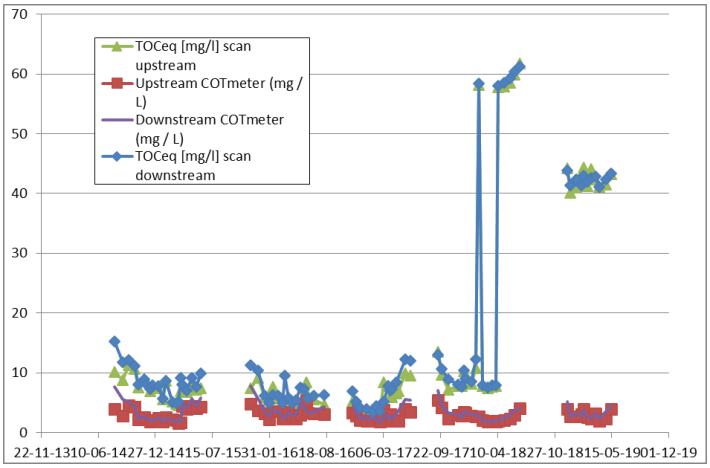

Figure 5: The TOC measured by NDIR and SCAN for upstream as well as downstream measured through the year 2013-2018.

As seen from the graph above (figure 5), there is a significant difference between the two, TOCeq SCAN and TOCeq LAB measured for upstream and downstream; except a steep peak for TOCeq SCAN downstream. This high peak is due to the deposition of the carbonate on the lenses, which needs to be cleaned frequently. Cleaning with the water is not sufficient as residues are left behind. By performing Mann & Whitney test, it is found that TOCeq LAB and TOCeq SCAN have different distribution (rejecting the hypothesis H0: parameter A&B have similar distribution) with alpha=5% risk at both inlet and outlet. The same results are obtained for the year 2016-17 and 2017-18.

2.3.4 GAS Measurement: CO_2 and CH_4 :

After measuring carbon in the sediment, vegetation and water compartment, we need to focus on gas compartment. In order to quantify the amount of carbon released and uptake by the wetland, it is important to understand the carbon dioxide and methane fluxes. We know that Carbon Dioxide is released via respiration and absorbed via photosynthesis phenomenon. It is difficult to quantify entirely the carbon fluxes as it involves complex pathways. Similarly, Methane is emitted via ebullition, diffusion and plant mediated transport; however carbon is constantly being transformed throughout this complex process. To understand this below is the analysis of the same:

2.3.4.1 Quantification of Carbon Dioxide:

A: Eddy Covariance data collection:

Eddy covariance flux tower is placed at the site. Data is continuously measured on the site for one year 1st January 2017 to 31st December 2017. The lake station is equipped with an Infra-Red Gas Analyzer (IRGASON © Campbell scientific) for CO_2 and H_2O, a 3D sonic anemometer (IRGASON © Campbell scientific), and an air temperature/humidity probe (IRGASON © Campbell scientific). IRGASON © Campbell scientific: Open-Path mid-infrared absorption gas analyzer integrated with a three-dimensional sonic anemometer. The sensor were located on the tower at 2.5m above the surface, as to include a large source area without the footprint extending outside of the wetland for most of the time. 3D wind speed, CO_2 and H_2O concentrations were recorded at 10 Hz. Data were collected on CR6/1000/3000/5000 data logger (Campbell scientific) and were sent in real time for processing and storage. Additionally, metrological measurements were collected continuously and stored on data logger (Campbell scientific) once every 30mins. The collected high frequency data were filtered and despiked to remove the noise using standard EC processing method via EdiRe Software (Campbell scientific). For more information on the IRGASON © Campbell scientific can be found on https://www.campbellsci.com/irgason and for EdiRe software https://www.geos.ed.ac.uk/homes/jbm/micromet/EdiRe/Tutorials/.

B: Footprint Analysis:

We used a footprint model to determine the distribution of the origin area of each 30 min aggregated flux observation. The footprint model uses wind speed, wind direction, boundary layer stability, surface roughness and turbulence data in order to trace the probability that a certain parcel of air that was measured at the flux tower top originated from any specific point at the land surface. Footprint maps provided the detailed spatial distribution of the different land cover type, in this case lake and forest. The footprint model was generated using Quantum GIS based on GPS surveys and remote sensing images (Google Earth). A 2D footprint likelihood matrix within each half hour was spatially integrated across all points either lake or forest to determine the probable percentage of that reading that originated from each. This work for our study was done by the team of scientist from University of Estonia. Reported carbon dioxide fluxes were restricted to the wind directions 100° to 270°.

Footprint model uses wind speed, wind direction, boundary layer stability, surface roughness and turbulence data to trace the flux emerging from the any specific point at the land surface. Data from the eddy tower is continuously measured on site for one full year from 1st January 2017 to 31st December 2017. The lake station is equipped with Infra-red Gas Analyzer for CO_2 and H_2O measurement, a 3D sonic anemometer for wind velocity and an air-temperature/humidity probe. There is a pre-processing of high frequency data collected by eddy tower. During preprocessing data is filtered and despiked using the standard EC processing methods. The wind velocity measurement were rotated using planer fit method (Wilczak et al., 2001) and the Webb-Pearman-Leuning correction for open path instruments were applied (Webb et al., 1980). This methodology can be found in details in Duman et al., 2018.

2.3.4.2 Carbon Quantification of Methane Gas:

To measure the methane we have had four campaigns in 2014-2015. In May 2014, twelve chambers and an associated control systems were installed from wetland inlet to outlet and these have been operational for one year duration. Methane CH_4 is measured by the automatic chambers as well as via manual chambers placed at a different positions. Chambers measured 40x40cm and with height of 25 cm and a given volume of 40 L. There are 12 chambers placed in the wetland numbered from 1 to 12 as shown in the figure 6 below explaining the position of the chambers placed in the wetland. Eight chambers were anchored to the depth of 25cm in the shallow part of the wetland and four chambers to the depth of 80cm in the deeper part of the wetland. Methane emission were measured for 2-10 days in a month with sampling done at every 6 minutes, collecting high frequency data and which were repeated for all four seasons. Air from each chamber is pulled continuously by a pump through a CH_4 infrared gas analyzer (Cascade Quantum Laser Tildas © Aerodyne). The flow through the system is controlled by mass flow controller. Inflow and outflow rates were balanced in the chambers and internal pressure dynamics were examined. The samplings were done at every 6 minutes therefore sampling time would be 72 minutes/each chamber for 12 chambers. An individual chamber is selected every 1hr 12min. At 72 minutes the lid closes; CO_2 concentration is sampled every 6 minutes; that will give us 20 measurements per day per chamber.

Figure 6: Topography of the automatic chambers placed in the wetland numbered from 1-12 in yellow boxes.

Methane fluxes were measured by the automatic chamber methodology during the four campaigns held in 2014-15. The four campaigns were summer wet i.e. May 2014, winter i.e. November, spring i.e. March 2015 and summer dry i.e. October 2015. Twelve different chambers were placed in various place

with varying depth. Every month samples were taken from 2 up to 10 days with sampling done at every 6 minutes. Automated chambers have been used for measuring methane fluxes in various studies, similar methodology can be found in Goodrich et al., 2011.

3: Results

3.1 Sediment Storage

The Sediments account for ~4% carbon sequestration of which ~87% is inorganic carbon. This high inorganic carbon content can be explained due to the leaching of carbonate from the agricultural soil, and getting stored in the wetland. Zone 4 has highest carbon content, particularly inorganic carbon coming from the crop area by drainage. This area is conducive to sediment accumulation because of deeper bed with low speeds. Not only volume of the zone but water velocity plays an important role in sedimentation of fine particles. On the other hand, zone 5 is more turbulent since it receives the flow of entry from the collector ditch. The organic carbon values can also be explained by the density of the plants (Reed), the presence of trees all around the basin and the presence of microorganisms. When they disappear, and when the trees lose their leaves, this organic matter constitutes a substantial annual carbon supply. During the last 8 years plants have been able to establish their life cycle and provide significant amount of natural debris during their renewal which contributes to 0.47% of organic carbon in zone 4 and 0.44 % in zone 5.

The results of the laboratory analyzes make it possible to extrapolate masses of carbon at the scale of each zone, knowing their surface area, the volume and the weight of the sediments (Table 2, Table 3, Fig.7).

Tables 1, 2 and 3 shows the main results obtained by the zone on the site of the Rampillon. The set of calculated values is then aggregated across the whole wetland, giving total carbon mass stored in the sediments. (Table 3).

Table 1 : Total particulate carbon in sediment samples in March 2018

	C organic (%)	C inorganic (%)	C tot particulate (%)
ZONE 1	0.837	3.829	4.666
ZONE 2	0.675	3.861	4.536
ZONE 3	0.718	3.906	4.624
ZONE 4	0.466	4.033	4.499
ZONE 5	0.435	3.440	3.874

Table 2 : Carbon Mass estimates in the Rampillon wetland sediments by Zone in March 2018

	Zone 1(OUTLET)	Zone 2	Zone 3	Zone 4	Zone 5(INLET)
Sediment height (m)	0.35	0.16	0.10	0.18	0.21
Surface zone (m^2)	940	1210	850	1550	790
Volume Wet sed. (m^3)	329	194	85	279	166
Sed. weight Dry for 1 m^3 (kg / m^3)	212	688	734	772	639
Dry Sediment weight per area (kg)	69,799	133,209	62,344	215,248	106,083

Sed. weight Dry / m² (kg / m²)	74	110	73	139	134
Carbon Particulate Total / m² (kg / m²)	3.46	4.99	3.39	6.25	5.20
Carbon Particulate Total kg	3,257	6,042	2,883	9,683	4,110
Carbon Organic Particulate kg	584	899	448	1,002	461
Carbon Inorganic Particulate kg	2,673	5,143	2,435	8,681	3,649

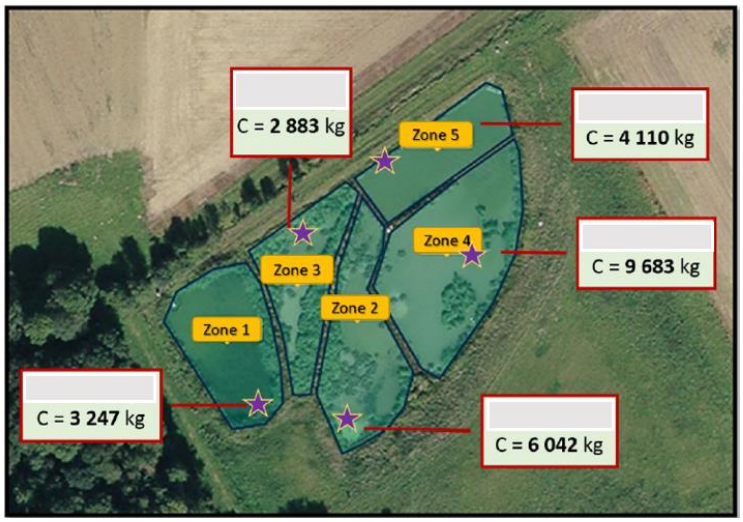

Figure 7: Aerial view of wet buffer zone with carbon mass extrapolated by zones

Table 3: Balance of Immobilized Carbon Masses in sediments as of March 2018

Carbon Mass of Sed.in kg	25,974	4.6t of Total Carbon each year for 2.5cm of sediment layer	2.3t of Total Carbon each year for 1.3cm of sediment layer
Organic Carbon Mass in kg	3,393(13%)	0.09 kg/ m^2 each year	0.05 kg/ m^2 each year
Inorganic Carbon Mass in kg	22,581(87%)	0.75 kg/ m^2 each year	0.36 kg/ m^2 each year

The total mass of carbon contained in the sediments estimated at 15 March 2018 is 25,974 Kg (Table 3). This value is representative of a state at a given date but in the case of sediment it is the result of an accumulation since the creation of the buffer zone in 2010 because there has never been a cleaning operation of the site. Based on the observed heights and the 8 years of existence of the zone, the rate of accumulation of sediments does not appear homogenous from one zone to another. It is on average 2.5 cm / yr. with values ranging from 4.4 cm / yr. in zone 1 to 1.25 cm in zone 3. This heterogeneity is not surprising since the tracing operation conducted at Rampillon (29/03/2017) showed that the movement of water within the basin are not characterized by the same flow. The speed in zone 1 is lowest and allows more time for sediments to accumulate. The data allow to put 2018 results into perspective by establishing the temporal dynamics of the sediments, even if the protocols are not strictly identical. The graph in figure 8 shows average values of carbon during last two years campaigns. The amount of total particulate carbon present in the sediments in ~ 4% in 2018 with slight increase from the previous year. This can be justified by the accumulation rate of sediment added to the bed every year. As 2.5 cm/yr of sediment is added every year. 0.09 Kg/ m^2 of Carbon Organic is sequestered by the wetland each year or 4.6T of total carbon is added each year to the wetland.

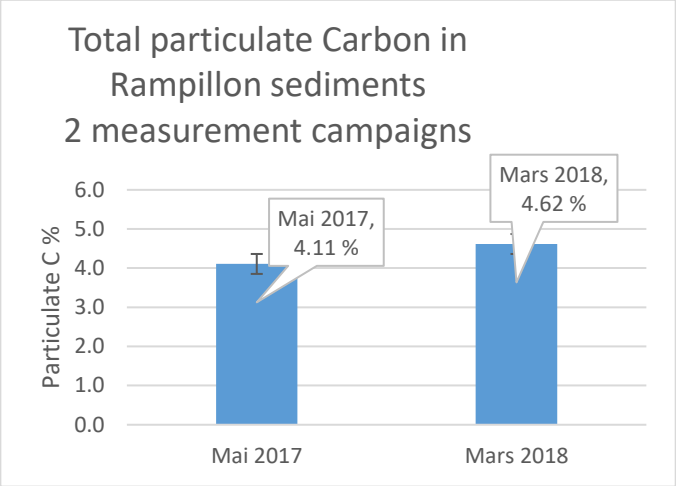

Figure 8: Total particulate carbon measured during the 2 measurement campaigns of 2017-218.

3.2 Storage by Vegetation

The vegetation has an important role in capturing *o*f carbon, which is absorbed by the vegetation through photosynthesis and then is transformed to the sediments as well as forms the part of detritus which is then returned to the atmosphere by the action of microbes. The plants harvested in the area are the most abundant and most representative of the vegetation of the site. *Phragmites Australis* (Identified by the letter P), *Juncus Inflexus* (J), *Carex Riparia* (C) and *Callitriche Palustris* (Cal) were collected in mid-May 2018. The numbers associated with the sampling points located on the map (Figure 4) were kept for better understanding. The vegetation surface area is measured by the drone and GIS animation, wetland area 6323.38 m^2 and vegetation is 2444.85 m^2 which results in 38.66 % of total wetland covered in vegetation.

Species	Nomenclature	C organic (%)
P (Roseau)	**P1T**	43.310
	P1rhiz	41.445
	P1R	36.265
	P3T	43.090
	P3rhiz	40.885
	P3R	39.765
J (Jonc)	**J1T**	43.910
	J1R	39.515
	J2T	42.780
	J2R	34.925
C (Carex)	**C1T**	42.970
	C1R	35.640
	C3T	43.115
	C3R	39.590
	C3 Flowers	46.115
Cal (Calitriche)	**Cal2**	37.345
	Cal3	35.350

Table 4: Carbon content in dry plant samples. T denotes Trunk and R denotes the Roots and Rhizomes. C3 has flower heads that were also analyzed at from the rest of the plant. May 2018.

The results of the analysis (Table 4) shows that the organic carbon of the samples is significantly higher than for the sediments. It is important to specify that all the plants are not at the same stage of growth,

which impacts their respective carbon contents. The Sedge (*Carex*) and the Rush (*Joncus*) are in the flowering stage while the Reed (*Phragmite*) is still in the growth stage. It is therefore in a phase where it captures more carbon to ensure its development which could explain these higher values. In the case of organic carbon, the values are more homogeneous although they are also lower in the roots.

Table 5: the plants were not sampled on equivalent areas to ensure representative intermediate densities for each species.

	C		P		J		Cal	
	Carex C1	**Carex C3**	**Roseau P1**	**Roseau P3**	**Jonc J1**	**Jonc J2**	**Calitriche Cal 2**	**Calitriche Cal 3**
Surface / cm²	552	323	90	150	625	425	240	255
Fresh weight (g)	1691.4	1968.9	675.8	1050.7	1670.4	322.9	48.4	145.0
Dry weight (g)	552.3	423.5	216.3	270.6	399.8	70.2	4.0	13.5
Water reduction (%)	67	78	68	74	76	78	92	91

The plants were dried before being weighed fresh but the values overestimate the weight in water due to dampness of the roots which were difficult to dry (Case of J1). The reduction in water of the four species remains important, since their dry weight represents less than 35% of their total mass. These four species are typical of wetlands, and it is not surprising that their biological structure is predominantly water. A drone photo dated 23/05/2018 serves as a support for determining the areas occupied by each species. From the densities, weights and C contents of each sample, the total stock of immobilized carbon is determined. The results of Table 4 and 5 are reported at the scale of the whole wetland (Table 6, Fig 9-10).

Table 6: Results of spatial analyzes and estimates by species 2018

	C	P	J	Cal
	Carex	**Roseau**	**Jonc**	**Callitriche**
Surface area ZTHA m²	821	856	548	220
Total Trunk weight (kg)	0.780	2.503	0.714	0.076
Total root weight (kg)	8.791	15.501	1.491	-
Mean Trunk % C	43.043	43.200	43.345	36.348
Mean Root % C	37.615	38.015	37.220	-

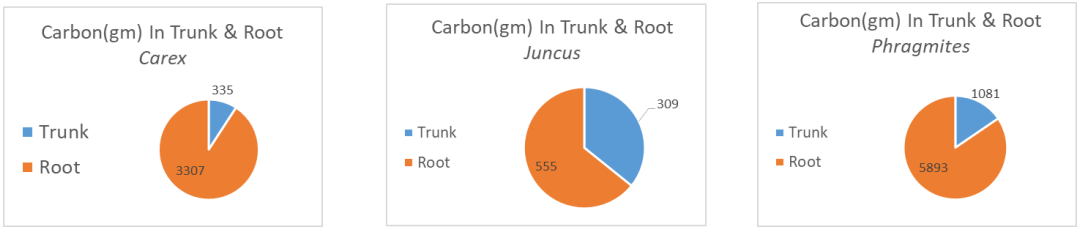

Figure 9: Total carbon masses in Kg in each macrophytes species at the scales of the whole basin

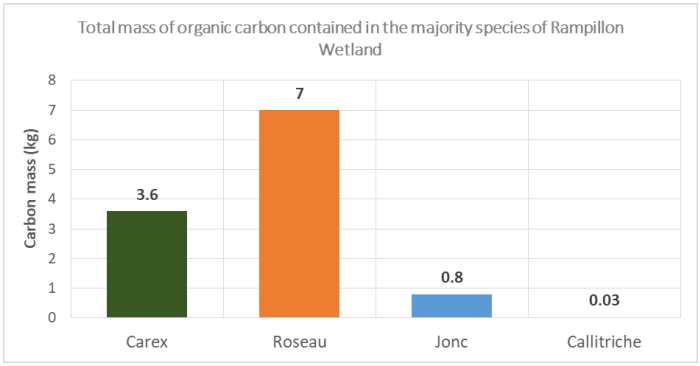

Figure 10: Total mass of organic carbon contained in the dominant species of Rampillon wetland

Several points emerge from the estimates above (Figure 9&10). First of all, a valid observation for all species concerns have large biomass (represented by large trunk & root weight). Reed stores the most carbon, about 7Kg in the basin. These measurements were carried out while the growth of the plants was not yet complete and the selected samples are of the intermediate density. The reeds are denser along the edges of zones 1 and 4, which suggests that the values found are underestimated compared to reality. Even if we increase the volume of the total vegetation biomass by double, the amount of carbon sequestered will be very less compared to the sediment sequestration.

3.3 Carbon Balance by Water at INLET/OUTLET:

3.3.1 Carbonate Inorganic Carbon:

The pH of the soil is ~7.2 which is near neutral. We found from the sediment results that it mostly consists of inorganic carbon. As per the distribution diagram below figure 11, with the soil pH of 7.2 the contribution of carbonate and bi-carbonate as follows:

$[CO_2][CO_3^{2-}] = 10^{-4}$ mol/kg

$[HCO_3^-] = 10^{-2}$ mol/kg

We can conclude the distribution of soil consisting of 80% inorganic and 20% organic carbon. This is amount of inorganic carbon is coming from the discharge of the water or the drainage water from the agricultural field and inorganic carbon gets leached into the sediment. We know that the carbonate and bi-carbonate can be converted into carbon dioxide as well as can react with the dissolved carbon dioxide in the presence of sunlight and this reaction is highly controlled by the pH of the water. Slight disturbance of the pH can trigger the equilibrium and this will affect the relative concentration of carbonate and bi-carbonate ions in the soil. We have observed increased amount of methane as well as increased amount of carbon dioxide which can be due to the excess carbonate and bi-carbonate in the soil. This hypothesis needs to be investigated further to establish the argument along with checking the stability of the sediment.

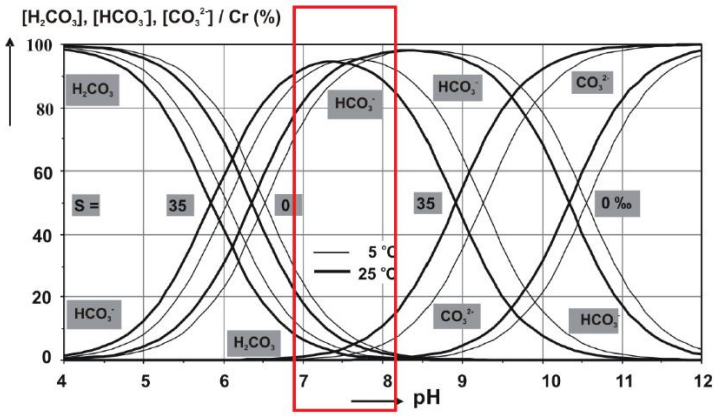

Figure 11: the distribution fractions of carbonic acid and percentages of carbon content total.

3.3.2 Total Carbon: TIC/TOC

As seen in the methodology, we measured the Total organic carbon value from SCAN probe at the site as well as SCAN installed from the LAB. In order to understand if there is difference between the two methodologies, if there is any, they differ by what value. To find out this we calculated flow weighted mean concentration of the TOC at the in situ flow SCAN value; these details regarding the same can be found in the Appendix 4. The SCAN probe makes it possible to determine a value of DOCeq and TOCeq from the total absorption spectrum at a time t as a function of a reference signal. As for the flow, the TOCeq concentrations obtained by the field SCAN probe ranges from the 0.95 mg/L to 25.06 mg/L for

the year 2016-17 and 5.58 mg/L to 52.97 mg/L for the year 2017-18. While the laboratory values vary within the range of about 3.44 – 12.30 mg/L for the year 2016-17 and 7.64 – 60.32 mg/L for the year 2017-18 respectively. The value differs by the factor of magnitude 2 for the 2016-17 as for the year 2017-18 SCAN in lab recorded higher value of TOCeq than the SCAN field. The difference between the TOC measured by SCAN on field and SCAN in lab vary by the average of 1.005 mg/L for the year of 2016-17. Similarly, the difference between the TOC measured by SCAN on field and SCAN in lab for the year 2017-18 vary by 1.264 mg/L.

It is possible that the calculation of TOCeq SCAN is overestimated due to the interference with the suspended solids and other dissolved and particular compounds during the high discharge flow. However, uncertainty about the validity of the high concentrations measured by the SCAN probe makes it difficult at this stage to decide between the relationships obtained by the different methods.

Due to this specific reason, as shown in the figure 12 a&b; for quantifying TOC we have high frequency measurement whereas for TIC we use bimonthly flow weight samples to analyze the total carbon.

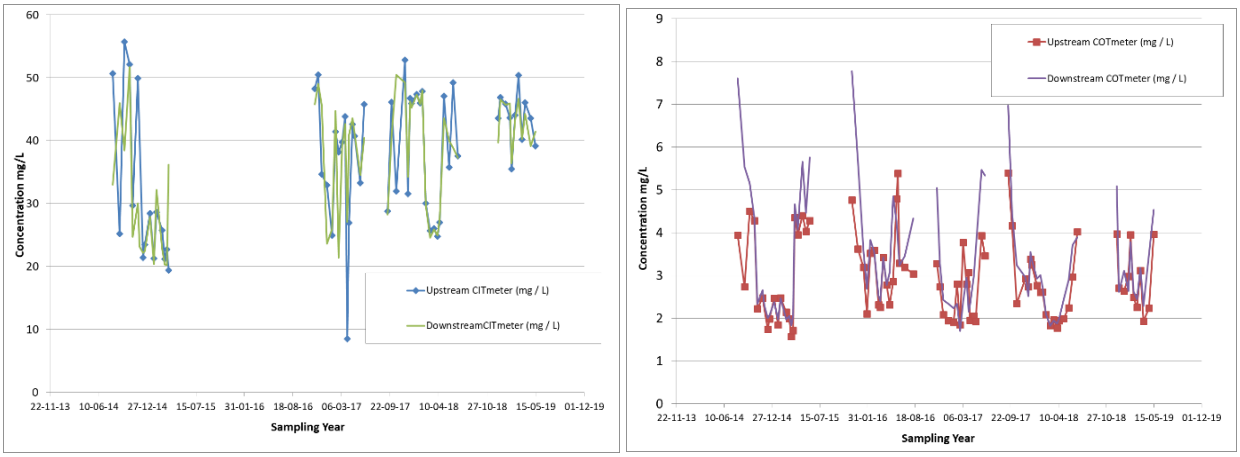

Figure 12:a&b the TIC & TOC measured at the upstream and downstream in the LAB from the point sampling method respectively from 2014-2018.

Figure 12 shows, TIC LAB upstream and downstream measurement do not show much difference in concentrations except few days as well as TOC LAB upstream and downstream shows same concentration at the lab. In fact, there is no clear conclusion that can be withdrawn between the TOCeq values obtained by the probe monitoring continuously or manually for better understanding of the phenomenon at the drainage during the high flow events. It is quite possible that the automatic probe greatly overestimates the concentrations of TOC but this also cannot be excluded that laboratory measurements may be underestimating the TOC by default sampling during the high flow events.

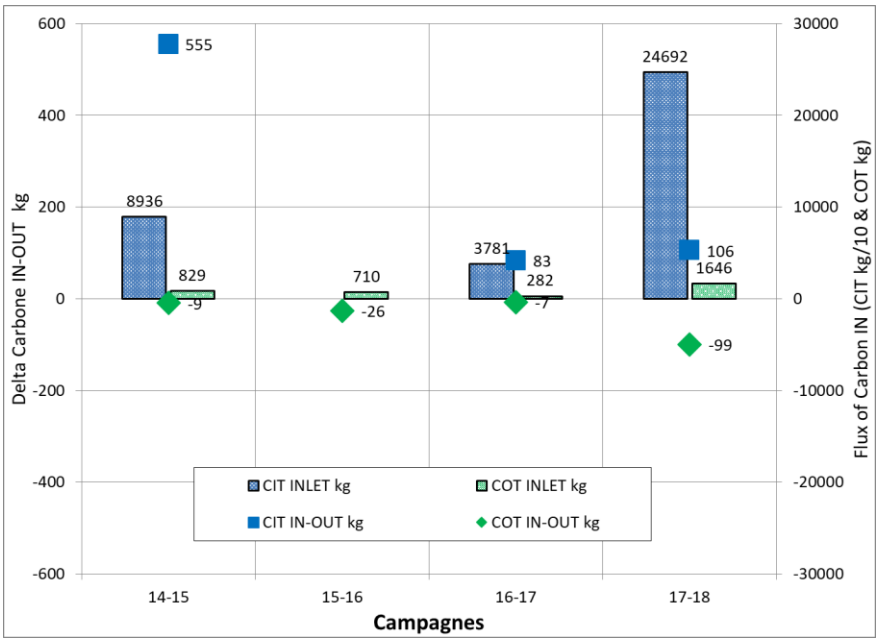

Figure 13: the result of the campaigns done from 2014-15 to 2017-18 to study the flux of carbon flowing IN and OUT of the wetland.

Until now we were trying to understand the flow behavior of TIC/TOC in situ and lab measured directly from the samples either via SCAN probe or water sampling. Now we are trying to focus on the total carbon organic and inorganic sequestered in the wetland. We calculated the flux generated by the TIC/TOC for the given volume of water flowing through the outlet probe. Summary of the calculations is given below.

Table 7: the result of the carbon flux generated in the wetland from the year 14-18

year	Drainage Flow Volume m3	CIT IN kg	COT IN kg	CIT IN-OUT kg	COT IN-OUT kg	CIT IN-OUT %	COT IN-OUT %
14-15	360081	8936	829	555	-9	6%	-1%
15-16	219115	-	710	-	-26	-	-4%
16-17	101979	3781	282	83	-7	2.2%	-2.5%
17-18	630972	24692	1646	106	-99	0.4%	-6%

From the table above we can see that in the year 2014-15 TIC at the inlet was 8936 kg and at the outlet was only 555 kg which means there is much more inorganic carbon getting deposited in the sediments in the wetland. Similarly for the year 2016-17 3781 kg was input and at the outlet only 83 kg was measured; for the year 2017-18 24692 kg was the input and at the output was 106 kg only, high value at the inlet suggests flooding event occurred in the region. There is more inorganic carbon getting leached to the sediment. Now, look at the TOC values, it suggests that the organic carbon are more in the outlet than in the inlet. TOC in 2014-15 at inlet was 829 kg and outlet was more by 9 kg, in 2015-16 inlet was 710kg and outlet was 26 kg more organic carbon, 2016-17 282 kg was the inlet and the outlet has 7 kg more and in the year 2017-18 1646 kg was in and out has 99 kg more of organic carbon. The percentage of sequestration for inorganic carbon is 6% for the year 2014-15. Similarly, 2016-17 it was 2.2% and in 2017-18 it was 0.4%. We can conclude that there is more organic carbon at the outlet than the inorganic carbon resulting due to microbial activity and algae bloom. Furthermore, there is leaching of inorganic carbon in the sediment by ~6% which forms the part of ~87% inorganic carbon found in sediments.

3.4 Carbon dioxide:

Figure 14 shows the annual cycle of CO_2 fluxes in a year 2017, using only observed data. The data shows strong seasonal variations with maximum emission occurring in the winters and maximum uptake happening during the period from spring to summer.

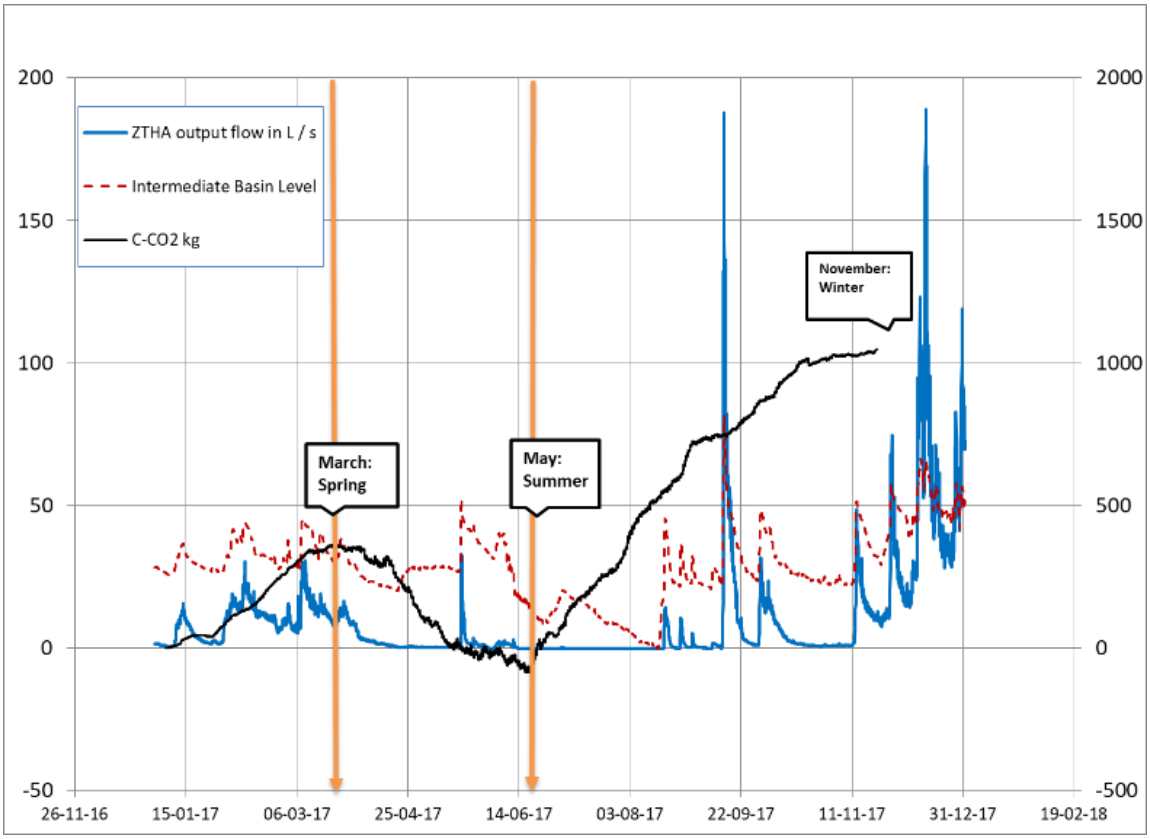

Figure 14:the graph measuring carbon dioxide with the intermediate basin level i.e. water level in the wetland and discharge of water in the wetland as ZTHA output flow

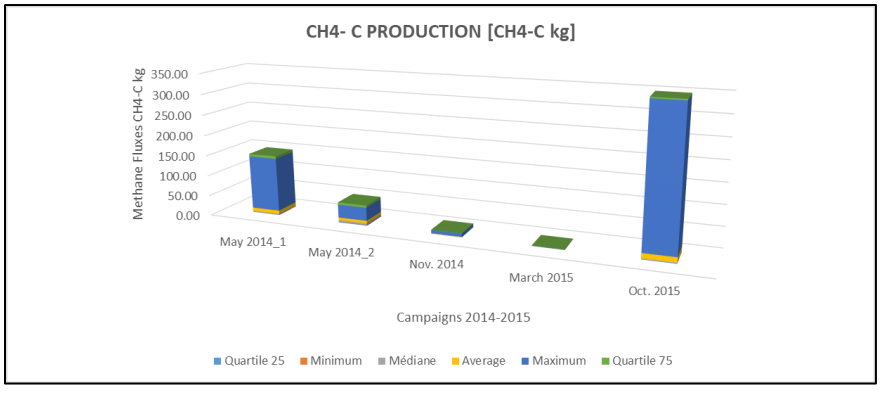

Figure 15: the means, average along with lower and upper quartile range of the methane flux from the wetland during the year 2014-2015.

Carbon flux is measured for the duration from 1st January to 31st December 2017. Maximum carbon is sequestrated in wetland occurs during the growing period of the vegetation i.e. from March to May whereas there is an emission of carbon flux from the wetland during the winter i.e. completion of growth period for vegetation. Net Carbon uptake in the wetland is ~500 C- CO_2 kg per year and ~1000 C- CO_2 kg or 1T of Net Carbon emitted per year. We must keep in mind that this uptake of carbon includes the respiration of microorganisms which are still present during the growing season. Moreover, we can say that the Net Carbon Uptake of ~500 C- CO_2 kg is underestimated due to the fact net value of negative CO2 is equivalent to difference between plant photosynthesis and microbial CO_2 respiration also known as Net Ecosystem Exchange (NEE). Furthermore, one can see clear relation between discharge of the wetland and carbon emission, as emission is increased during the high drainage flow i.e. during the winters, precipitation is high due to rainfall events (figure 14).

We observed that consistently high rates of CO_2 production when soils were saturated and anoxic before starting to decline after dry conditions sets in the December. It is useful to note here that during winter and high rain event wetland were completely anoxic as evidenced by CH_4 production figure 15 particularly in the month of October.

3.4.1 Climatic Variation:

Obvious seasonal patterns were observed for carbon dioxide *and the precipitation* in the wetland. Air temperature changes very smoothly, increasing from January to August, and then declining until December. Nearly half of the annual rainfall occurs from November to January. From March to May, the time period for plant re-juvenescence and growth, the precipitation usually is sufficient. The relatively low amount of precipitation from May to June in 2017 generated a comparatively dry climate for wetland plants, which may have exerted the productivity and therefore, altered the gas fluxes.

3.4.2 Seasonal Variation of GHG budget:

The apparent seasonal pattern appears as a sinusoidal shape with vertex occurring around March and November and lowest peak occurring around May and early January for ecosystem respiration CO_2 (Figure 14). Actually, the monthly CO_2 for wetland start decreasing from Mid-March when the plants growth begins, and peaks when plant biomass reaches its maximum. For the whole growing season (March to May) CO_2 fluxes account for ~ 16 % of the corresponding annual flux. In the non-growing season, the CO_2 fluxes are higher that corresponds to the rainfall event and changes in the temperature. For ecosystem respiration, wetland shows one peak following the seasonal pattern of the plant growth and two peaks in the non-growing season, the soil respiration still contributes to the ecosystem respiration when autotrophic respiration is negligible(SONG et al. 2009). For CO_2 wetland acts as a source in non-growing season and sink in the growing season.

The yearly pattern of fluxes over the period 1st January to 31 December 2017 are shown in the figure 14 for eddy covariance measurements. The points represents the mean value of measurements for the half hour ending at that time. Their peak uptake at the height of their growing season in late May were around – 1.656 mg/ m^2/s.

Higher temperature resulted in reduced CO_2 uptake by vegetation. This effect may have been due to drying out of the vegetation or completion of their growth period rather than a direct response to temperature. Possible reason for increasing the CO_2 uptake over the study period can be hypothesized. First, wetland temperature increased through the period and this may have promoted enhanced vegetative activity, particularly for rooted vegetation. A second possibility was increased uptake due to

increase in extent of green vegetation. The wet black hollows, which were covered with water at the beginning of the period, gradually became drier and by the end of the study were covered with the fresh vegetation.

3.4.3 Overall estimates:

During the observation period of 01/01/2017 to 31/12/217, the wetland emitted the CH_4 & CO_2 at the ecosystem level. The majority of the gas released occurred in non-growing seasons in which CH_4 and ecosystem respiration CO_2 were 12.60 CH_4 -C kg and 700.05 C- CO_2 Kg respectively. For the specific year, the ecosystem acted sink for CO2 during the growing period and source for the non-growing season. Wetland ecosystem consumed 28.95 C- CO_2 Kg in 2017(negative carbon fluxes) this sink is mainly contributed by growing season consumption of gases.

3.5 Methane Gas discussion:

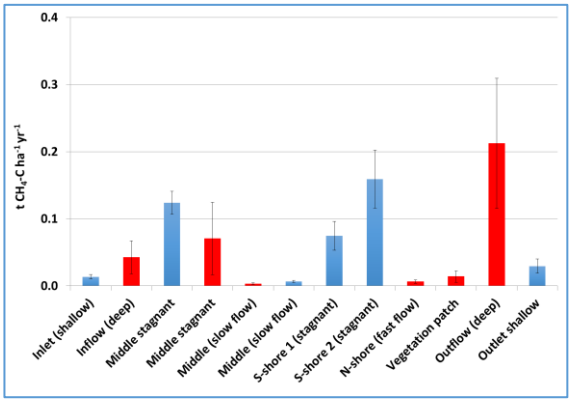

Figure 16: Methane measured at the different points on the wetland with varying water depth; Red Bar: Manual Chamber Blue Bar: Automatic Chamber

The figure 16 shows the results of the CH_4 emission from the different parts of the wetland. Methane was measured over the four campaigns held in the year 2014-15 covering two campaigns summer, one in winter and one in spring. We can see that the deeper part of the wetland have higher emissions than the shallow depth of wetland. Higher emissions from the deeper part shows the probability of the sediment height and water table being related to the CH_4 emission fluxes.

3.5.1 Diurnal Variation

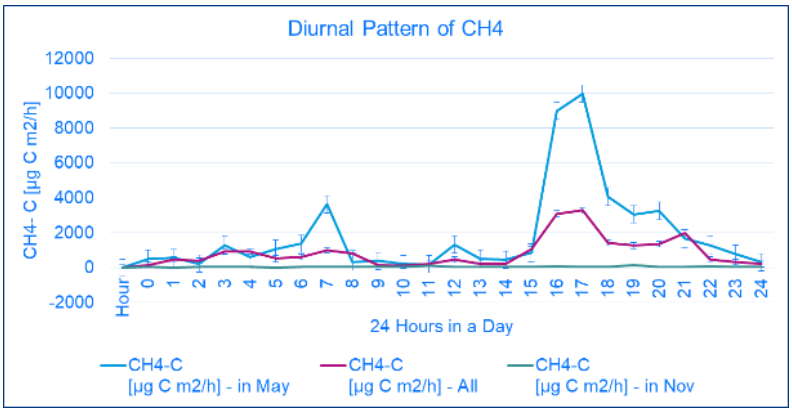

Figure 17: the diurnal pattern of the methane emission measured during the whole year 2014-2015 with the measurement taken during 24 hours a day

Figure 17 shows the diurnal pattern of the methane emission over the whole year measured for 24hours a day. Strong diurnal cycles were present both during the winter and during the summer seasons, though the summer pattern is much more pronounced due to overall stronger fluxes (3-5 times stronger than the winter). The peak day time flux rates in both the seasons were roughly 2.5 times higher than the nighttime fluxes. Data at night is highly variable, it is important to note that while daytime measurements may have over 100 half hours contributing to the daily average curves, most nighttime's half hours have fewer 50 measurement due to stable atmospheric conditions. Therefore, a small number of relative extreme observations can result in a much higher standard deviations and mean for the night time averages, which supports the results from the paper by T.H.Morin et al., 2014.

The Diurnal variation patterns of CH_4 fluxes through the three transport pathways as shown in the figure 18 can be concluded as follows: Diffusion, Plant Meditated Stomatal Conductance and Ebullition figure 19.

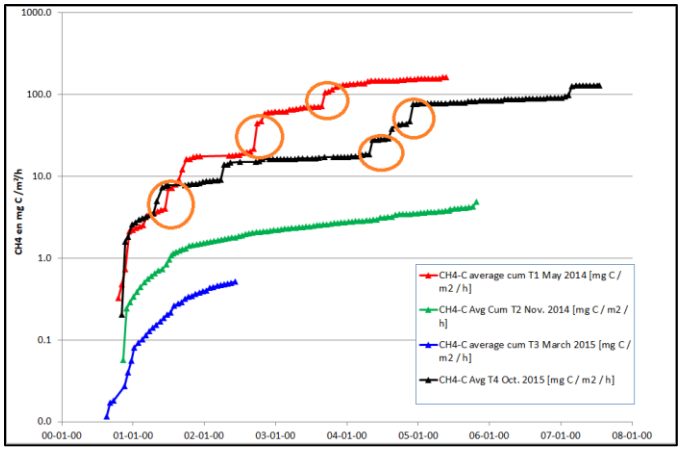

Figure 18: the emission of methane during the whole year with relative to four seasons i.e. summer, early winter, winter and spring. This graph shows the ebullition and diffusion process occurring in the wetland. Red and Black lines shows ebullition and diffusion whereas Green and blue shows only diffusion.

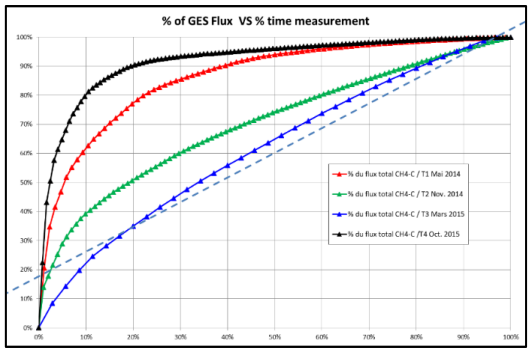

Figure 19: the percentage of gas measured relative to the number or percentage of sampling time. Similar to figure 18 here black and red have maximum/ abrupt emission during the largest amount of time compared to November and March.

3.5.2 Transport Pathways for Methane

Several methods have been employed and characterized the magnitude of ebulative release as well as the frequency with which it occurs (Coulthard et al. 2009), and reported rates range from 0-35,000 mg CH_4 / m^2/day , though typical values are on the order of ~ 1000 mg CH_4 / m^2/day (Bartlett and Harriss 1993; Comas and Slater 2007; Tokida et al. 2007). Results obtained for the magnitude of ebullition measured at Rampillon is CH_4 -C 145.76 mg C/ m^2/h in the month of May 2014 when measured for 6 days. We measured ebullition as an abrupt and sudden release of the gas marked by steep slope shown in the figure 18, which are more during the summer i.e. May 2014 and early winter i.e. October 2015. During the month of May 2014, ~60% of the total methane fluxes were released during the 10% of the total time it was measured (figure19). Abrupt ebullition were captured by the chambers but the small and highly frequent and short duration gas bubbles are difficult to capture in chamber measurement. Mean ebulative methane emission in May was 2.632 mg C/ m^2/h and in October it was 2.367 mg C/ m^2/h.

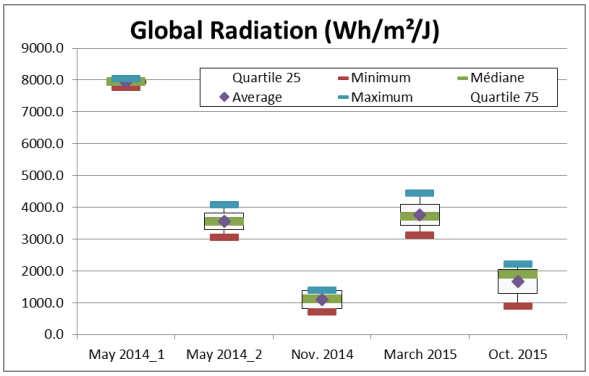

Figure 20: the global radiation absorbed by the wetland. May has two summer period; early summer and late summer.

As the methane fluxes is mainly due ebullition, this phenomenon is affected by many external and abiotic factors such as wind temperature, air temperature, air- water surface temperature, sediment temperature due to solar irradiation. We can also see that from the figure 20, the radiation keeps varying throughout the year which in return influences the sediment temperature, water-air temperature and amount of carbon dioxide captured by the plants rate of evapotranspiration.

3.5.3 Statistical Analysis:

We did PLS to find out the contribution different parameters such as pH, nutrients, organic matter supply and seasonal variations. Previously we performed PCA analysis but it did not result any satisfactory correlation so decided to perform PLS on the entire data sets. PLS was performed due to the properties of PLS model which is well suited when the matrix has more variables than observations and when there is multicollinearity among the variables. PLS on the complete data set to find the contribution of each parameter on the methane emission with the Pearson Correlation method. For the entire data set, the first two t components explain the contribution of them to the CH_4 and N_2O parameter marked in green table given below.

Table 8: the results of PLS done on the methane matrix data. To understand the percentage contribution of each component in the methane flux. Matrix of correlation of the variables with the components t:

Variable	t1	t2	t3
pH KCl	-0.239	0.260	-0.241
N %	0.593	-0.235	0.602
NO3-N mg/kg	0.372	-0.201	0.239
NH4-N mg/kg	-0.021	-0.044	0.020
P mg/kg	0.330	0.192	0.100
K mg/kg	0.036	0.243	0.518
Ca mg/kg	0.536	-0.100	0.445
Mg mg/kg	0.607	-0.448	0.145
OM %	0.604	-0.253	0.568
DN mg/kg	0.090	0.205	0.184
DIC mg/kg	-0.491	-0.050	-0.149
DOC mg/kg	-0.293	0.726	0.454
Time-01/05/2014	0.356	0.878	0.154
Time-01/11/2014	-0.792	-0.156	0.544
Time-01/03/2015	-0.083	-0.287	-0.644
Time-01/10/2015	0.520	-0.434	-0.054
CH_4-C µg C m^2/h	0.150	0.584	0.016
N_2O-N µg N m^2/h	-0.771	-0.164	0.344

Colored pink in the table 8, DOC is positively correlated with t2 and negatively correlated with t1. Time 01/05/2014 is positively correlated with both t1 and t2. Time 01/11/2014 is negatively correlated to both t1 and t2. Time 01/03/2015 is also negatively correlated to both t1 and t2. Time 01/10/2015 is positively correlated on t1 and negatively correlated on t2.

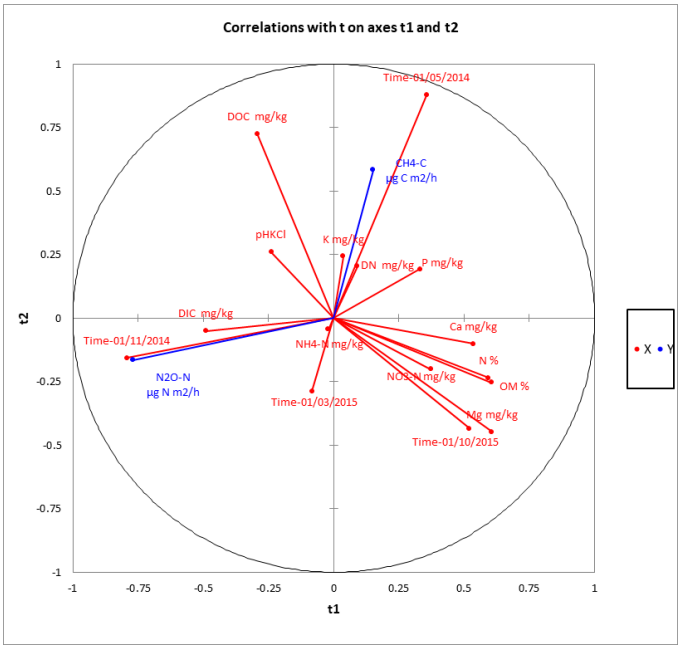

Figure 21: scatter plot of CH_4 and N_2O on the set of observations on axes t1 and t2.

The same is been confirmed by the scatter plot Figure 21, where Y is the observations defined by CH_4 and N_2O from the data set and X is the set of variables namely pH, Time, Nutrients etc. On the scatter plot it is evident that DOC and Time 01/05/2014 are more closely correlated to CH_4 whereas pH, DN, Ca NO3-N etc. are not highly correlated. Similarly Time 01/10/2015 is negatively correlated to CH_4 .

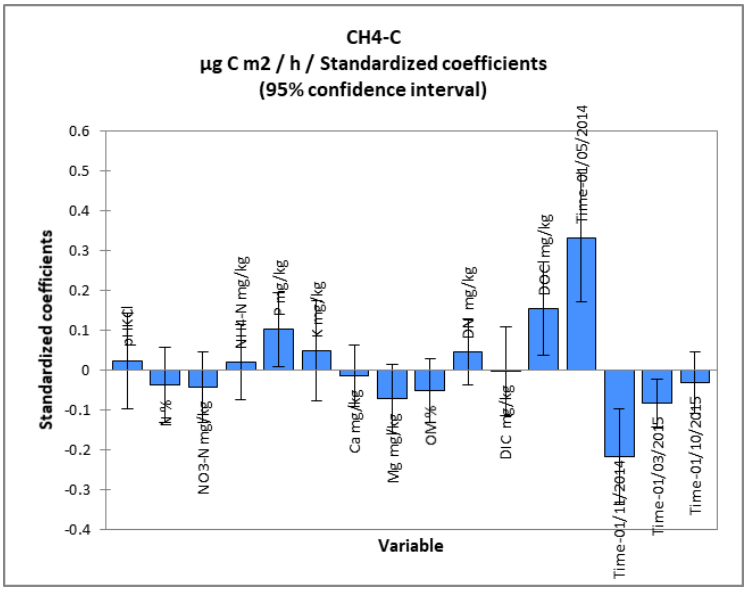

Figure 22: shows the graphical representation of normalized coefficient for methane emission

After doing PLS on methane it is found that DOC has a higher impact on methane than any other parameter figure 21 & 22. The correlation coefficient is a measure that determines the degree to which two variables' movements are associated. The most common correlation coefficient, generated by the Pearson product-moment correlation, may be used to measure the linear relationship between two variables presented in the figure 22. There is a visible seasonal effect on the methane emission especially during the summer and early winter. As DOC is having positive coefficient of correlation 0.153 in the PLS model with -0.293 t1 & 0.726 t2 distribution; it has a positive impact on the methane mission, which is in congruence with the DOC/TOC results. There is more DOC/TOC present in the wetland at the outlet than inlet. This organic carbon is due to organic matter result of microbial activity and presence of algae and algae bloom due to nitrogen and nutrient rich discharge from the agricultural field.

Time has both positive as well as negative correlation on t1 and t2 with CH_4 emission. We can see that from the PLS, summer period i.e. May is positively correlated with 0.356 t1 & 0.878 t2 distribution and has a positive correlation coefficient 0.332. Similarly, late winter i.e. November and spring i.e. March both are negatively correlated with distribution -0.792 t1 & -0.156 t2; -0.083 t1 & -0.287 t2 with negative correlation coefficient as -0.218 and -0.083 respectively (Table 8). This shows that methane emission is greatly impacted by the seasonal affect. There is considerable season influence on the methane emission from the wetland which should not be ignored while calculating the methane budget. This support our extrapolation which projected seasonality to play an important role in methane budget. This seasonal effect is due to higher solar irradiance and water-air temperature and microbial activity.

4. Discussion

4.1 Carbon Sequestration in Sediments

The carbon cycle represents a complex structure with the carbon being continuously transformed and transported to different compartments. The analysis of all compartment at the local scale produced the result as 25,974 Kg of static total carbon trapped in the sediments is due to 8 years of accumulation, which means a storage of 3,393 Kg Corg. The dynamics of the inorganic carbon 22,581 kg is more questionable. This amount of organic carbon that is found in the sediments as result of degradable matters and microbial action as well as drainage water incoming from the agricultural runoff. Based on the observed height and 8 years of existence of zone, the rate of accumulation of sediments does not appear homogenous from one zone to another. As we already saw earlier ~2.5cm of sediment layer is accumulated every year and sediment corresponds to only ~4% of total carbon. As 2.5 cm/yr of sediment is added every year, 0.09 Kg/ m^2 of Carbon Organic is sequestered by the wetland each year or 4.6t of total carbon is added each year to the wetland. Regardless, another scenario needs to be taken into the account; as Rampillon is a constructed wetland an additional layer of soil approx. ~10 cm was added at the initial construction phase. For the carbon sequestration calculation this layer needs to be rejected; we need to subtract 10cm from height of the sediment measured from each zone. This gives us 1.3cm of sediment layer added per year, which contributes to 2.3t of total carbon sequestered, and 0.05 Kg C/ m^2 organic carbon sequestered in the wetland. Moreover, not only sediment height and volume but water velocity is conducive to the sediment settlement: turbidity places an important role in the sediment influx. As we know that the turbidity is higher at the INLET than at the OUTLET, more sediments flows into the wetland which in return, generates more suspended solids in the zone. The efficiency of the wetland to retain inflowing sediment and C and the resulting rates of deposition, are dependent upon several actors including wetland size, land use, design, sediment load and water influx. In agricultural landscapes, constructed wetlands have been shown to serve as important sinks for C

through their retention of sediments and associated OM (Johnston, 1991). We can say that 2.3t of total carbon with 1.3 cm of sediment layer added every year is an overestimation due to the influencing factor like turbidity and suspended solids with moreover Rampillon behaves like a sink for carbon given high carbon stored annually. These findings are consistent with other studies evaluating the efficacy of constructed wetland to improve the water quality in agricultural landscapes (Johnston, 1991; Braskerud, 2001; Jordan et al., 2003; Maynard et al., 2009).

4.2 Carbon Sequestration in Vegetation

Vegetation has an important role in capturing *o*f carbon, which is absorbed by the vegetation through photosynthesis and then is transformed to the sediments as well as forms the part of detritus which is then returned to the atmosphere by the action of microbes. The total amount of carbon sequestered by the vegetation is very less compared to the sediments even when they cover ~ 38% of the wetland. The amount of total organic carbon stored by the vegetation needs to be related to the life cycle of the each species. On the one hand, root storage is not renewed in its entirety every year, and can be considered as a static stock whose value is not taken in to consideration in the annual dynamic report. These measurements were carried out while the growth of the plants was not yet complete and the selected samples are of the intermediate density. The reeds are denser along the edges of zones 1 and 4, which suggests that the values found are underestimated compared to reality. Total carbon captured by the vegetation is only 10 kg in comparison to 490 kg of organic carbon by sediment every year. Carbon storage by sediments are higher than the uptake by the vegetation, even if the volume of the vegetation is doubled. These finding of our study wetland is not in line with many other similar studies on carbon stored by vegetation such Maynard et al., (2011); Brinson et al., (1981) where wetlands are also highly productive ecosystem capable of producing and incorporating large amount of biomass into the soil.

4.3 Carbon Sequestration in Water

For the carbon sequestration by water we are measuring the values of total organic and inorganic carbon at the INLET and OUTLET of the wetland. To support this, we did bimonthly flow weight analysis for TIC to analysis the inorganic carbon and performed continuous high frequency measurement for TOC by SCAN probe installed at site as well as in the LAB in order to understand if there is any difference between the two methodology and if they differ by what value. The difference between the TOC measured by SCAN on field and SCAN in lab vary by the average of 1.005 mg/L for the year of 2016-17. Similarly, the difference between the TOC measured by SCAN on field and SCAN in lab for the year 2017-18 vary by 1.264 mg/L. It is possible that the calculation of TOCeq SCAN is overestimated due to the interference with the suspended solids and other dissolved and particular compounds during the high discharge flow.

It is evident from table 7 that there is a sequestration of inorganic carbon, coming as agricultural drainage into the wetland, but the percentage is not so much and this inorganic carbon gets leached to the sediment which contributes to the storage of carbon in sediment. The percentage of sequestration for inorganic carbon is 6% for the year 2014-15, 2016-17 it was 2.2% and in 2017-18 it was 0.4%. By taking the mean of the amount of TIC flux difference at the outlet, which is found to be 248kg of TIC added every year since the year 2014-15. This is an annual increase in the sediment layer added each year so the wetland gets replenished every year with 2.3±0.248t of Total Carbon. We need to keep in mind the uncertainty in the TIC concentration due to missing data from 08 Oct 2018 to 20 May 2019. On the contrary, there is additional TOC present at the OUTLET; 1% in 2014-15, 4% in 2015-16, 2.5% in 216-

17 and 6% in 2107-18. This additional amount of carbon present in the water at the outlet of the wetland which can be the result of microbial activity and presence of algae/algae bloom due to nitrogen and nutrient rich discharge from the agricultural field. The source of carbon in sediment comes from detritus of the freshly added organic matter, decomposing of old organic matter by microbial activity, solar radiation convert dissolved carbon dioxide to carbonate, soil profiles often been leached of carbonate rock, inherited dissolved carbonate and algae. The contribution of these factors were not explored in this study. For the carbon balance of water does not quite gives any satisfactory results which is evident by TIC/TOC value being almost equal at INLET and OUTLET.

4.4 Carbon Dioxide: CO_2

Carbon Dioxide is measured with the Eddy tower installed at the site and footprint model is used to determine the origin area of 30 min aggregated flux observation. Using footprint model and vegetation map we can analyze the data by vegetation cover. When examining the annual NEE after gap filling, at the study site, the vegetation cover served as a sink for CO_2 during the summer i.e. March- May or growing season of 2017. At the same time, there was microbial respiration still present which we did not account for. The fluxes during the winter or non-growing season i.e. July-February started as net sources of CO_2 in 2017.

From the figure 14 we can see that most of the CO_2 uptake occurred in summer, where photosynthesis increases with higher temperature but CO_2 emissions in winter are more complex to understand. Our summer measurements of CO_2 show that the average rate of ecosystem CO_2 uptake was 0.012912 mg / m^2/s or average Net uptake of 135.3 kg C- CO_2 during the 4 months of growing season (March to June) and winter measurements of CO_2 show that the average rate of ecosystem emission was 0.847 mg/ m^2/s or average emission of 700.05 kg C- CO_2 during the non-growing winter season (July-February). The factors contributing to winter release of CO_2 and CH_4 include both biological and physical mechanisms. Biological processes are uncertain, but studies have shown that microbial decomposition of organic matter can occur below the freezing point, as low as -16℃ (Clein and Schimel, 1995; (Panikov and Dedysh 2000). Root respiration may also contribute to the CO_2 flux in winter (Grogan et al. 2001). In addition to production of recently fixed carbon, winter respiration may include the decomposition of older pools of soil organic matter (Trumbore and Harden 1997; Winston et al. 1997; Goulden et al. 1998).

Our results are in congruence with the findings of these papers, CO_2 was emitted throughout the winter period, not just the during the warmest part of the season with maximum CO_2 emission occurring during the winter season. The highest uptake were observed when ground was at higher temperature>0℃ indicating that photosynthesis is only occurring when plant tissues are above freezing and when there is sufficient light for photosynthesis. We can say that there is higher photosynthesis occurring during the summer growing season and higher respiration happening during the winter. Using C14 measurements in black spruce forest, (Winston et al. 1997) reported that a larger fraction of CO_2 flux in winter, compared with summer, originated from decomposition of older carbon stored in depth in the soil rather than from recently produced carbon from root respiration.

When considering the entire CO_2 budget, respiration only accounts for the loss of CO_2 to the atmosphere, not the uptake by plants. Photosynthesis rates might also increase with a warmer climate, but the measured magnitude of respiratory losses in this study were far greater than plant uptake during the study period. For 2017, our ecosystem respiration CO_2 in non-growing season accounted for ~84.8 % of the annual budget. The non-growing greenhouse gas budget in natural wetland was ignored in some previous studies (Yang et al., 2006). Our study supports the conclusion that the non-growing gas

fluxes can never be ignored (Melloh and Crill 1996; Treat et al. 2007). Comparing summer and winters fluxes it showed a persistent pattern: winter fluxes higher than the summer fluxes during the entire research study resulting wetland being a source of CO_2 releasing ~1000 kg of C- CO_2 into the atmosphere.

4.5 Methane Gas:

The quantification of carbon becomes difficult as it evolves to take into account various other interlinked processes. Emissions are the net result of CH_4 production in the anaerobic zone minus the oxidation of some fraction of the CH_4 as it moves from the wetland soil to the atmosphere; CH_4 transport occurs by diffusion through the soil matrix, diffusion through the plant aerenchyma that bypasses the soil matrix, and the subsurface CH4 bubble movement and release or ebullition (Whalen 2005). Carbon transported to the sediments and to the organic matter after their senescence is further degraded by the microbes which in turn is transported back to atmosphere by the action of ebullition and advection. In particular, ebullition has been difficult to quantify due to highly stochastic nature of this flux pathway. In our study, methane emission is mainly done by ebullition events ranging from CH_4 -C 0-550.7 mg C/ m^2/day which is in the reported range by the (Coulthard et al. 2009); (Bartlett and Harriss 1993; Comas and Slater 2007; Tokida et al. 2007) from 0-35,000 mg CH_4 / m^2/day.

4.5.1 Transport Pathways:

Diffusion is a linear process controlled by water table, sediment temperature and water temperature; which occurs mainly in November and March i.e. late winter and spring during the study period shown in figure 18. Diffusion through the profile refers to the movement of methane up through soil and bodies of water to reach the atmosphere. It has less sudden impact on the methane emission compared to ebullition which is the major contributor of CH_4 to the atmosphere. It contributes to only ~11% of the total methane emission during our study period whereas ebullition contributes to ~89.4% of total methane emission. Diffusion occurs considerably in winter and spring i.e. November and March with less consistence during the summer and early spring i.e. May and October.

The Ebullition methane fluxes presented more complex diurnal variations as in figure 17. The ebullition methane flux was generally higher during daytime and lower at night; however, in May, the ebulative methane flux tended to reach the peak value after the dusk. The day and night variations of CH_4 fluxes are mainly related to the following reasons: (1) the plant mediated transport is restrained due to high relative humidity at night, while it is enhanced by the low relative humidity during the day. (2) During the day, the sediment temperature is higher and results in lower carbon threshold values; thus the ebullition is increased. The situation is opposite at night. In particular, ebullition has been difficult to quantify due to the highly stochastic nature of this flux pathways.

In particular, wind speed, water-air temperature, sediment temperature, turbulence may affect surface layer peat methanotrophy by rapidly re-oxygenating surface peat pore spaces (Kimball and Lemon 1971). It is important to note that ebullition may also occur as a steady stream of relatively small bubbles, which would result in a linear increase of chamber headspace CH_4 concentration over the time (Coulthard et al. 2009). Thus the ebullition data presented here may be less than the total ebullition. We have shown that ebullition event are not necessarily rare release of CH_4 from depth, triggered by overburden pressure events or subsurface buildup beneath confining layers; rather ebullition represents a regular flux pathways for CH_4 as typical as diffusion and plant transport.

4.5.2 Interannual Variation

Diurnal variation patterns shown in figure 17 of CH_4 emissions are directly regulated by the changes in water table, water depth, sediment temperature, solar radiation and relative humidity. Moreover, the patterns are also indirectly regulated by the salinity, pH and redox potential. These factors regulate diurnal variations by influencing the methane production and then the methane concentrations; thus their effect on diurnal variation pattern are more complex and inexplicit.

The methane data obtained by the automatic chamber can be extrapolated for each day of the year obtaining the seasonal pattern of the methane emission. The seasonal cycle of methane suggesting that while most emissions are during the summer, but the winter fluxes are not negligible for example during CH_4 -C 0.011 kg C/ ha/d in November but October has mean of CH_4 -C 0.216 kg C/ ha/d emission rate. The strong seasonal and diurnal cycles of methane may indicate a potential bias of manual measurement campaigns and particularly those based on short seasonal campaigns. Further studies needs to be done for longer period of measurements. As per the (Mitsch et al. 2013) in the model simulation of atmospheric budget calculated over the period of 300 years for seven temperate and tropical wetlands suggests, all wetland eventually cause net decrease in CO_2 equivalent in the atmosphere and the maturation of the wetland plays an important role in deciding whether the wetland is a source of sink of carbon dioxide. As Rampillon is still in the young age of its life cycle, it is acting as a source of methane with 40.85 CH_4 -C kg releasing into the atmosphere per year caused mainly due to 87% via ebullition and methanogenesis by microbial activity.

4.6 Carbon Budget:

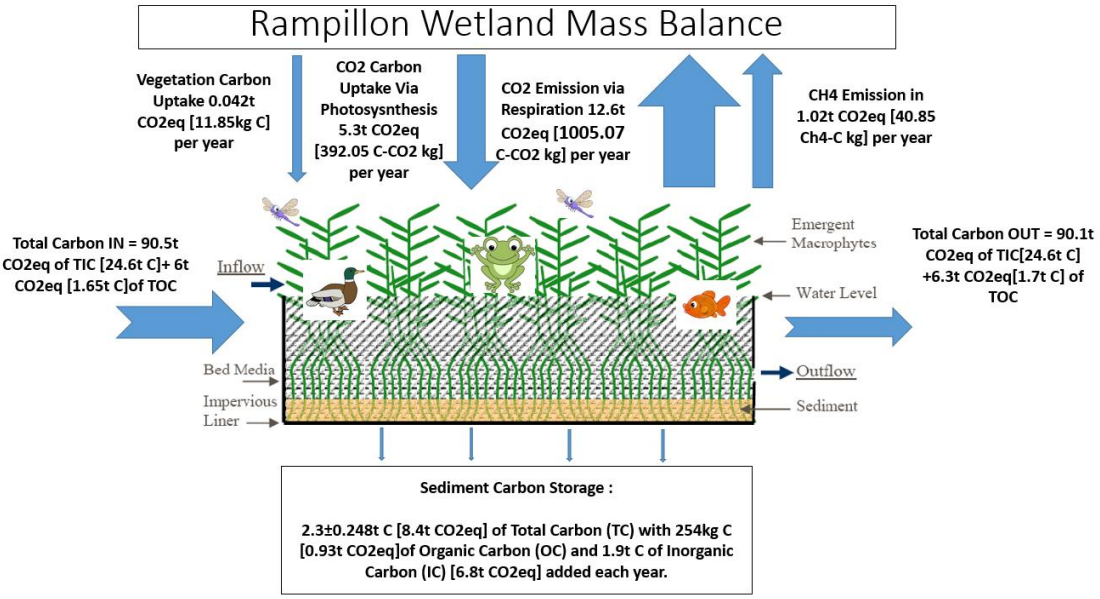

Figure 23: Rampillon wetland Carbon Mass Balance comprising of all the compartments of the artificial wetland in C- CO_2 kg equivalent. Arrows representing the difference in the fluxes percentage.

4.6.1 Net Gas Budget: Carbon Dioxide CO_2 and Methane CH_4

Long term efficiency and GHG emission potential of an artificial constructed wetland are still unknown. Our study aims to fill this gaps by studying the gas fluxes. After calculating the carbon mass balance of Rampillon wetland, it is evident that there is a strong emission of CO_2 and CH_4; a potent greenhouse gases. For the budget as calculated in figure 23, CO_2 is mainly emitted due to respiration accounts for

12.6t CO_2eq [1005.07 C- CO_2 kg] as well as 1.02t CO_2eq [40.85 CH_4-C kg] of methane was emitted with ebullition being the highest contributor. The percentage of carbon emitted is not compensated by the vegetation uptake as the accumulated biomass is very less, making wetland a source.

4.6.2 Net Carbon Budget:

In our study site we explored all the different compartments of wetlands; sediment, vegetation and water apart from the gas fluxes mentioned above. On studying the carbon balance diagram, we can conclude that even though the gas fluxes emission rate is high the amount of carbon stored in the sediment is higher contributor to carbon sequestration. Sediment sequestrating 8.4t CO_2eq [2.3±0.24t C] total carbon every year with 16.6t CO_2eq [4.6t C] being already stored in the wetland in due course of 8 years. "CO_2eq" is a very useful term for a number of reasons: it allows "bundles" of greenhouse gases to be expressed as a single number; and it allows different bundles of GHGs to be easily compared (in terms of their total global warming impact). Thus the system functions as carbon slight sink.

5. Conclusion

In our study, we saw during the analysis of sediment of the Rampillon wetland, it contains 87% of inorganic carbon which is supplemented by 248 kg of carbon each year through the discharge from the crop field. In comparison to sediment carbon sequestration, vegetation did not play very important in contrast to many other studies; which found important links between the vegetation and the carbon storage. During our study period, vegetation only contributed to 10 kg of carbon storage compared to 2.3t of carbon sequestered annually by sediments. This amount of carbon stored in the sediment can be overestimated due to turbidity factor influencing the sedimentation rate. We found more organic carbon at the outlet of the wetland due to enhanced microbial activity and algae bloom in the wetland, which was measured by the SCAN spectrophotometer probe. The wetland acted as sink for inorganic carbon but the percentage of carbon sequestration each year is not high. We used Eddy Covariance method to determine the CO_2 fluxes generated by the wetland, although there was not a simple relationship between CO_2 emission and temperature over the full range of ground temperature, there was significant correlation between winter temperature and net ecosystem exchange across the wetland. There is relationship between net ecosystem exchange and above ground plant biomass implies that the plant activity are important in explaining the range of CO_2 emission. Photosynthesis is the reason for carbon uptake but the measured magnitude of respiratory losses are far greater than the plant uptake. Wetland in acting like a source of CO_2 due to higher respiratory losses during the winter season releasing up to ~1t of C- CO_2 kg into the atmosphere. In our study, we used automatic chamber measurements technique to understand the methane fluxes from the wetland. We show a strong seasonal and diurnal pattern of methane flux. We demonstrate that these consistent temporal cycles must be accounted for in limited-time campaigns that measure methane emission only during some seasons or times of day. Ebullition played a major role in methane emission, with diffusion been ~11% of total methane flux. Previous studies have reported that plant mediated transport was the largest source of methane during the season (Walter and Heimann 2000; Wania, Ross, and Prentice 2010). However, our study did not explore this possibility of transportation pathways for methane. Further efforts should target measurements that could be used to parametrize such model to work on exploring all contributing factors. We can conclude in keep all these compartments in mind; that Rampillon a constructed wetland is still in young age of maturation, and a major source of CO_2 [1005.07 C- CO_2 kg/yr] and CH_4 [40.85 CH_4–C kg per year] throughout the year. As reported by (Mitsch et al. 2013); wetlands are the major contributors of GHG in their young age i.e. 13-15 years but they become radiative sink in the span of 100-300 years.

When calculating the complete wetland carbon mass balance as shown in the figure 23, Rampillon is a Net Sink of Carbon with storage in sediments playing significant role with 2.3t±248 kg total carbon added annually each year; only 10 kg of carbon sequestrated in vegetation not much compared to sediment; Total Inorganic and Organic carbon measured in the water at the INLET and OUTLET did not yield any significant amount for carbon sequestration; Carbon uptake due to photosynthesis is only of 392kg C- CO_2 in comparison to 1005.07kg C- CO_2 emitted due to winter respiration in one full year and finally 40.85kg CH_4 -C of methane emitted annually mainly due to ebullition contributing ~89% to total methane emission.

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Annexes

APPENDIX 1: SAMPLES AND ANALYZES OF SEDIMENT FROM THE RAMPILLON BUFFER ZONE, MARCH 2018.

Field protocol

The sampling points are chosen according to several criteria below:

- Aymeric Drouet's thèses on the Eco Dynamics and bio-availability of metallic contaminants in the artificial buffer zone of Rampillon (2015).
- Depending on the practical possibility of accessing the place of sampling.
- According to the preferred lines of current.
- In this context, 5 strategic points are determined.

The protocol and the precautions taken are based on the recommendations of the continental sediment sampling and pretreatment guide for physio-chemical analyzes of the WFD (Scientific and Technical Program, final document, Schiavone S and Coquery M. April 2010).

For each sampling point:

- The water and sediment heights are measured.
- 3 Samples are recovered in the first 15 cm of depth, and 50 cm away from each other.
- The layer of organic matter on the surface is removed as much as possible.
- The samples are placed in sealed bags identified by the marker.

The transport of the sediments is carried out in a cooler, and their conservation is done in a cold room at 4 ° C.

Figures Appendix 1: Rampillon wet buffer zone, March 2018. Sediment sampling in Zone 2.

Laboratory analyzes - C Total

- The 3 samples from each sampling point are mixed and homogenized in the same container. Excess water is removed if necessary.
- They weighed wet, put in an oven at 105 ° C for 1 to 2 days and weighed dry before being ground.
- They are then passed to the elemental analyzer which gives the percentages of nitrogen and total carbon (organic and inorganic).

Laboratory Analysis - Organic C

To obtain the values of organic carbon contained in the sediments, it is necessary to perform a decarbonation operation on each sample.

It is a pre-treatment that requires an oven temperature between 40 and 50 ° C, then requires 2 days of handling before giving usable results.

The acid tech protocol for elemental analysis is given below.

Time of realization: 48h

- 1h cold attack
- 1night of decantation

- 1 to 1:15 centrifugation
- 1 night of drying

Reagent

HCL 2N

Protocol

- In a BOD bottle, weigh 0.5 g of dry ground and add 10 ml of HCL 2N.
- Put it for 1 hour on oscillating agitator.
- Add 40 ml of water and let settle one night.
- Discard the supernatant without touching the sediment! and transfer the sample into a weighed 50 ml FALCON tube. Perform washing by centrifugation for 10 minutes at 3000 rpm, 3 to 4 times (until obtaining a neutral PH).
- Discard the supernatant, put the centrifuge tubes with etching residue in a beaker and put in an oven at 50 ° C overnight.
- Let cool and weigh the tube with its residue.
- Recover the dry residue, grind it and store the sample in glass pillboxes.

Note: Do not forget to weigh the FALCON tube.

APPENDIX 2: VEGETABLE SAMPLES AND ANALYSIS FROM THE RAMPILLON WET STAMP REGION, MAY 2018.

The reference document used is the "detailed protocol for harvesting plant biomass in vegetated areas of rejection, Role of the plant compartment within the vegetated zone of the" basin "type of Marguerites (30)" dated 2015 (Guerreiro H Bertrin V., Coquery, Boutin C.).

Field protocol

The sampling is done by plants by measuring the aerial extent / ground-footing area except in the case of the Reed. We take the emerged parts and the submerged parts.

Following a pre-sampling and for practical reasons, we realize at different geographical locations:

- Jonc /Rush: 2 samples of small plants → 1 sample = 1 plant
- Carex: 2 samples → 1 samples = 1 plant
- Roseau /Reed: 2 samples → 1 sample = several plant
- Callitriche: 2 samples → 1 sample = 1 plant

This give us:

Number of samples = 2 (Emerged and Immerged biomasses) x 2 (points) x 3 (emerged species) + 2 (points) x 1 (submerged species) = 14 total samples

The main constraints in the field are:

- A low water level.
- Difficult accessibility of plants.
- The choice of plants representative of the species, its density on the site and in good health.
- Cleaning the plants (large amounts of sediment nested with the roots).

Appendix Table 1: Field Protocol for Callitriche.

Submerged Macrophyte → Callitriche
- Homogeneous vegetation zone - Measure the airborne area by the plant (string and meter) - If entanglement of plants: unravel as possible to harvest only one plant

- Cut the stems flush with the substrate and leave the root system in place.
    - Only harvested stems
- Empty up the water

Appendix Table 2: Field Protocol for the 3 emerged species

Macrophytes Emerged • Phragmites australis • Juncus inflexus • Carex riparia
<u>Emerging biomass:</u> Homogeneous vegetation zone. Measure the aerial surface taken by the plant (string and meter). Cut the stems about 15 cm above the surface of the water with pruning shears • Possible to cut the plant to put it in the bag Empty up the water (after rinsing)
Jonc: 2 plants + surface measure Carex: 3 plants + surface measurement Roseau: Quadras of given surface (size of shovel) + samples of Quadra seedlings. *Note the life stages of plants.*
<u>Biomass immersed:</u> • With a shear or a sharp spade: cut vertically deep in the ground to cut the roots and rhizomes. • Pull hard and continue digging and cutting in parallel with pruning shears. • In the case of root biomass blocked in the soil: push the shears to the maximum, cut the tissues, leverage and pull the stems to uproot. • Pre-clean with water from ZTHA before conditioning • Empty up the water

Plants are placed in bags in a cold room at 4 ° C and treated the next day to prevent maceration phenomena.



Figures Appendix 2: Phragmite sampling, surface survey and plant extraction, May 2018.



Figures Annex 3: Sampling of Phragmites, Rhizomes and Callitriche. May 218. The sedimentary mass on the roots of the Phragmites plant is several kilograms.

Laboratory protocol

Objectives: Weighing of fresh masses, dry mass and analysis of organic carbon.

The most difficult part is cleaning the plants to remove all the sediment and animal debris.

Fresh weight measurement

Appendix Table 3: Laboratory Protocol for Callitriche.

Submerged Macrophyte Callitriche
Need to be close to a water point • Prepare the drying socks: marker numbering + tare weight • Wash the plants: sieve over the sink and then a cleaning tub filled with tap water. Remove sediment debris and organisms • Rinse with distilled water for chemical analyzes • Wringer: plants washed to wring as many times as necessary • Fill the drying bins without packing to facilitate drying • Weigh (fresh weight + weight of tare obtained)

Appendix Table 4: Laboratory Protocol for the 3 dominant species.

Emergent Macrophytes • Common Reed: Phragmites australis • Rush: Juncus inflexus • Shore sedge: Carex riparia
Emerging biomass: • Prepare the drying socks: marker numbering + tare weight • Wash the plants: sieve over the sink and then a cleaning tub filled with tap water. Remove sediment debris and organisms • Rinse with distilled water for chemical analyzes • Cut the plants into pieces of about 10 cm (no need to separate stems and leaves here) • Wringer: plants washed to wring as many times as necessary • Fill the drying bins without packing to facilitate drying • Weigh (fresh weight + weight of tare obtained)
Immersed biomass: • Prepare the drying socks: marker numbering + tare weight • Wash the plants: sieve over the sink and then a cleaning tub filled with tap water. Remove sediment debris and organisms • Cut the plants + roots + rhizome into pieces of about 10 cm • Scrub and clean thoroughly to remove all sediment residues • Rinse / wash with distilled water for chemical analysis • To spin at a steady pace

Samples are left in the open air for a day before being weighed to limit the moisture associated with their cleaning during weighing.

Dry weight measurement

Samples are placed in an oven at 45-50 ° C for 1-2 days as needed. They are then weighed.

Analysis

- The carbon contained in the plants is only in organic form, to carry out decarbonation operations is not necessary.
- The treatment of the plants is then identical to that of the sediments. The difference is a selection of the parts of each sample that will be analyzed. The harvested plants sometimes have senescent or damaged parts which partially

constitute the dry weight of each sample. The most representative parts of the roots, stems, rhizomes and inflorescences are selected for the analyzer.

- These are the parts that are then crushed and analyzed.

APPENDIX 3: TOTAL ORGANIC CARBON ANALYZER FOR TIC/TOC MEASUREMENT

HBAN-Chemistry method:

TOC Analysis (NPOC):

The technique used is NPOC (Non-Purgeable Organic Carbon). After acidifying (2N HCl) the sample to pH 2-3, a carrier gas is bubbled to remove all the inorganic carbon. The sample is then introduced into a combustion tube containing a catalyst and heated to 680 ° C. The carbon contained in the sample is converted to carbon dioxide and transported by the carrier gas to the NDIR detector (Non-Dispersive Infra-Red) where it will be analyzed.

Analysis of CIT (IC):

The analysis is done directly in the syringe. After acidifying the sample to pH 2-3, a carrier gas is bubbled and all of the inorganic carbon is converted to carbon dioxide. The carbon dioxide is then transported by the carrier gas to the NDIR detector (Non-Dispersive Infra-Red) where it will be analyzed.

Excerpt from the manual "TOC-L":

8.1 Principles of Analysis

Two types of carbon are present in water: organic carbon and inorganic carbon. Organic carbon (TOC) bonds with hydrogen or oxygen to form organic compounds. Inorganic carbon (IC or TIC) is the structural basis for inorganic compounds such as gas carbonates and carbonate ions. Collectively the two forms of carbon are referred to as total carbon (TC) and the relationship between them is expressed TOC=TC-IC.

The principles underlying TC and TN analysis are explained in the following sections.

8.1.2 Principles of IC (Inorganic Carbon) Analysis

Two methods for measuring IC using the TOC-L are available: analysis within the injection syringe and analysis using the optional IC reactor. In both methods, the measured IC consists of carbon derived from carbonates, hydrogen carbonates and dissolved carbon dioxide.

Defining IC

The IC measured by TOC analysis consists of the carbon contained in carbonates and in carbon dioxide dissolved in water. By acidifying the sample with a small amount of hydrochloric acid to obtain a pH less than 3, all carbonates are converted to carbon dioxide (CO2) by the following reactions:

$Me2CO3 + 2HCl \rightarrow CO2 + 2MeCl + H_2O$

$MeHCO3 + HCl \rightarrow CO2 + MeCl + H_2O$

Carbon dioxide and dissolved carbon dioxide in the sample are volatilized by bubbling (sparging) air or nitrogen gas that does not contain carbon dioxide through the sample.

Analysis Using the IC Reaction Vessel (H Type Instrument)

The TOC-L IC reactor kit is used to sparge the IC reaction solution (acidified reaction liquid) with carrier gas. Sample is injected into the IC reaction vessel and the IC in the sample is converted to carbon dioxide, which is volatilized by the sparging process and detected by the NDIR.

Analysis With-in the Syringe (N Type Instrument)

The sample is acidified to pH 3 or lower in the syringe, using hydrochloric acid. The sample is sparged with carrier gas and the IC in the sample is converted to carbon dioxide and detected by the NDIR.

8.1.3 Principles of NPOC (Non-Purgeable Organic Carbon) Analysis

After acidifying the sample to pH 2 to 3, sparge gas is bubbled through the sample to eliminate the IC component. The remaining TC is measured to determine total organic carbon, and the result is generally referred to as TOC. However, in the TOC-L, this analysis value is referred to NPOC to distinguish it from the TOC value obtained by calculating the difference between TC and IC. NPOC stands for non-purgeable organic carbon and refers to organic carbon that is present in a sample in a non-volatile form. NPOC and TOC (obtained by IC elimination) described in the TOC-related standard methods and referred to in water quality-related test methods (JIS, ASTM, EPA, EN) are identical. Purgeable organic substances in the sample can be lost during the sparging process. Consequently, when the sample contains purgeable organic substances, TOC should not be measured by the NPOC method. If the dissolved purgeable organic component in the water sample is large, the amount volatilized during sparging is relatively small. Generally, the amount of purgeable organic substances in natural environmental, public and purified water is small; as a result, NPOC can be referred to as TOC.

APPENDIX 4: TOTAL ORGANIC CARBON: FWMC CALCULATIONS

Measurement of dissolved organic carbon

Several sources of data are available to us. Those made in the laboratory on water samples and other measured continuously with the SCAN probe.

Analysis of dissolved organic carbon in laboratory

The water sampled is filtered in the laboratory (GF / F filters with a porosity of about 0.5 µm grilled at 500 ° C for 4 hours to remove any organic matter from the filters). The solid particles present in the raw samples are thus separated from the dissolved fraction, the DOC being measured by the hot oxidation method by extractive gas analyzer. This method consists in oxidizing all the organic matter in CO2 by high temperature heat treatment (800 - 1200 ° C) (Vivarat-Perrin 2006). This CO2 is then measured by non-dispersive infrared sensor probe (NDIR).

SCAN probe analysis:

The S: CAN probe is based on a measurement of UV / visible absorption spectroscopy. It is based on the absorption capacity of the UV light of organic matter. The entire absorption spectrum of the solution over a wide range of wavelengths is measured. This spectrum reflects in a complex way the presence of nitrates, nitrites, anionic surfactants, organic matter dissolved colloidal and solid particles in suspension (Fig. 5). Using reference spectra for each of these classes of constituents, the signal is de-convolved by an algorithm that allows the calculation of the most probable concentration of each of these constituents (Thomas et al., 2007).

Flow-weighted mean concentration

Equals the load for a given time period divided by the product of the volume of streamflow for the period (∑Qt) and a unit's conversion factor (k).
For the calculation of the FWMC, data on the concentration, sample time window and flow are required for each sample. The equation for calculating the FWMC is

$$FWMC = \frac{\sum_{1}^{n}(c_i * t_i * q_i)}{\sum_{1}^{n}(t_i * q_i)}$$

where q_i = flow in the i^{th} sample

Source: water quality laboratory, Heidelberg College

With this equation the concentration in each sample is weighted by both the time and the flow that accompanied it. The FWMC represents the total load for the time period divided by the total discharge for the time period. The ratio of FWMC to TWMC indicates whether a pollutant tends to increase in concentration as flow increases. If the FWMC>TWMC, that pollutant, on average, increases with increasing flow. Figure 1

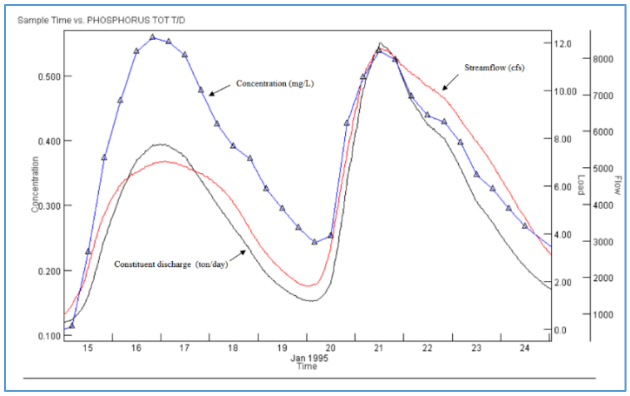

Figure 1 shows the relationship between concentration, discharge and flow.

		TOCeq [mg/l]	DOCeq [mg/l]	Compound TOCeq value from the scan & Q calculation	Ratio(Comp TOCeq/TOC eq)
16/11/2016	RAV 161116	6,894	4,781	0,95	0,138
		6,894	4,781	0,95	0,138
29/11/2016	RAV 291116	5,195	3,308	10,18	1,960
		5,195	3,308	10,18	1,960
14/12/2016	RAV 141216	4,103	2,546	4,94	1,204
		4,103	2,546	4,94	1,204
05/01/2017	RAV 050117	3,964	2,3	-	-
		3,964	2,3	-	-
26/01/2017	RAV 260117	3,435	1,952	-	-
		3,435	1,952	-	-
09/02/2017	RAV 090217	4,426	2,614	3,29	0,743
		4,426	2,614	3,29	0,743
22/02/2017	RAV 220217	3,544	2,113	1,47	0,415
		3,544	2,113	1,47	0,415
07/03/2017	RAV 070317	5,156	3,319	4,07	0,789
		5,156	3,319	4,07	0,789
21/03/2017	RAV 210317	7,747	4,542	4,25	0,549
		7,747	4,542	4,25	0,549
29/03/2017	RAV 290317	6,969	3,775	3,32	0,476
		6,969	3,775	3,32	0,476
05/04/2017	RAV 050417	7,271	3,733	5,27	0,725
		7,271	3,733	5,27	0,725
20/04/2017	RAV 200417	8,372	4,634	8,62	1,030
		8,372	4,634	8,62	1,030
22/05/2017	RAV 220517	12,301	8,008	25,06	2,037
		12,301	8,008	25,06	2,037
07/06/2017	RAV 070617	11,941	7,476	23,85	1,997
		11,941	7,476	23,85	1,997
12/09/2017	RAV 120917	13	8,021	-	-
		13	8,021	-	-

		TOCeq [mg/l]	DOCeq [mg/l]	Compound TOCeq value from the scan & Q calculation	Ratio
27-09-17	RAV 270917	10.598	6.295	25.294	2.387
		10.598	6.295	25.294	2.387
18-10-17	RAV 181017	8.893	4.333	10.671	1.200
		8.893	4.333	10.671	1.200
22-11-17	RAV 221117	8.03	4.42	9.022	1.124
		8.03	4.42	9.022	1.124
05-12-17	RAV 051217	7.635	4.266	8.296	1.087
		7.635	4.266	8.296	1.087
13-12-17	RAV 131217	10.318	4.841	25.304	2.452
		10.318	4.841	25.304	2.452
19-12-17	RAV 191217	8.987	5.152	26.104	2.905
		8.987	5.152	26.104	2.905
09-01-18	RAV 090118	8.502	4.543	20.651	2.429
		8.502	4.543	20.651	2.429
24-01-18	RAV 240118	12.213	5.213	23.001	1.883
		12.213	5.213	23.001	1.883
02-02-18	RAV 020218	58.432	34.19	-	-
		58.432	34.19	-	-
16-02-18	RAV 160218	7.836	4.084	7.462	0.952
		7.836	4.084	7.462	0.952
06-03-18	RAV 060318	7.508	3.613	9.444	1.258
		7.508	3.613	9.444	1.258
21-03-18	RAV 210318	7.92	4.131	5.584	0.705
		7.92	4.131	5.584	0.705
05-04-18	RAV 050418	7.844	3.778	6.225	0.794
		7.844	3.778	6.225	0.794
13-04-18	RAV 130418	58.069	33.887	8.418	0.145
		58.069	33.887	8.418	0.145
02-05-18	RAV 020518	58.542	34.105	13.459	0.230
		58.542	34.105	13.459	0.230
23-05-18	RAV 230518	59.267	34.998	26.346	0.445
		59.267	34.998	26.346	0.445
08-06-18	RAV 080618	60.324	35.853	52.97	0.878
		60.324	35.853	52.97	0.878
27-06-18	RAV 270618	61.264	36.216	37.291	0.609
		61.264	36.216	37.291	0.609

Table 1 & 2 shows the TOC value from the SCAN probe in-situ and SCAN LAB respectively calculated through FWMC and their ratio to understand the difference between the two; which differs by the magnitude of 1 mg/L.

Year 2016-17	Year 2017-18
TOC Aveg	TOC Aveg
1.005	1.264