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PhD Thesis

Greenhouse gas (CO₂ and CH₄) net emission from freshwater wetlands with different primary producer communities

Supervisors:

Dr. Marco Bartoli

Dr. Daniele Longhi

Prof. Pierluigi Viaroli

Coordinator:

Prof. Giulio De Leo

Candidate: Cristina Ribaudo

**Ai miei genitori per gratitudine
e alla nuova arrivata Jezerca per augurio**

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Abstract

The present work focuses on the regulation of greenhouse gas emission by different primary producers in shallow freshwater wetlands. In eutrophic environments the increase of organic matter input to the sediment favours the shift of primary producer communities. As a consequence of the increase of water turbidity and phytoplankton growth submerged rooted vegetation is replaced by floating-leaved species. The latter are selected in nutrient-rich and light-limited aquatic bodies.

The main hypothesis of the present work is that the shift in plant composition and the prevalence of a community over another one trigger changes in water oxygenation and production of greenhouse gas (CO_2 and CH_4) in the sediment and thus the efflux towards the atmosphere. In this context, the presence of submerged aquatic vegetation plays a major role in maintaining water oxygenation and favour aerobic microbial processes (e.g. methane oxidation), whereas the physical barrier created by floating-leaved and free-floating macrophytes at the water-air interface supports anoxic conditions in the water column and in the sediment. As a main consequence, respiration processes and methanogenesis in the sediment are favoured, as well as the accumulation of methane and carbon dioxide in the water column.

To test these hypotheses, two laboratory experiments were performed to investigate the oxygen transport to the rhizosphere by a submerged rooted macrophyte (*Vallisneria spiralis*) and its subsequent effects on methane oxidation, carbon fixation and nutrients retention (Chapters 5 and 6). Secondly, an *in situ* experiment was carried out to define the role of a floating-leaved macrophyte (*Nuphar luteum*) in conveying methane from the sediment to the air through the aerenchyma and fixing carbon dioxide in biomass (Chapter 7). Finally, an *in situ* monitoring was performed over a range of shallow wetlands colonized by different primary producers, with the aim of investigating the effect of the colonization of free-floating macrophytes on water oxygenation and seasonal gas dynamics in water; theoretical greenhouse gas fluxes were also calculated (Chapter 8).

Results from the present study show that, following the shift of primary producers from submerged to floating forms, an increasing amount of greenhouse gases is released to the atmosphere. This outcome should be of global concern when considering the raising of aquatic environments that suffer from eutrophication and that are undergoing to regime shift; those environments are likely to be colonized by free-floating plants and significantly affect the global trace gas budget.

1. Introduction

1.1 Greenhouse gases emission from freshwater wetlands

Waterlogged and anoxic conditions make wetlands one of the major source of methane toward the atmosphere, contributing with a total emissions of 92 to 237 Tg CH₄ yr⁻¹, which is a large fraction of the total annual global flux of about 600 Tg CH₄ yr⁻¹ (Ehhalt et al., 2001; Solomon et al., 2007). Carbon emission from wetlands is characterized by a high temporal and spatial variability (Whiting & Chanton, 1992; van der Nat & Middelburg, 1998; Käki et al., 2001) and it is mainly regulated by the balance between primary production, respiration and decomposition processes, which are in turn strongly influenced by the typology of plants and their physiological status (Armstrong & Armstrong, 1988; Käki et al., 2001; Brix et al., 2001; Whiting & Chanton, 1993; Wetzel, 2006). Other primary controlling factors are the water table height and the temperature of air, soil and water (Segers, 1998; MacDonald et al., 1998; Wang & Han, 2005), as well as the presence of electron acceptors alternative to oxygen, such as sulphate, nitrate or iron, which can affect methane emission rates, especially when oxidation processes are enhanced by plants via high oxygen transport to the rhizosphere (Neubauer et al., 2005; Dodla et al., 2009).

Most studies on CH₄ fluxes from natural wetlands have focused on boreal regions, as these ecosystems are of importance for storing a large fraction of the global carbon pool in the soil, even if they occupy only a small portion of the earth's land surface (Buringh, 1984; Schlesinger, 1991; Gorham, 1991). Organic carbon accumulation in those environments is typically due to high productivity, elevated water tables and low decomposition rates (Gorham, 1991). Carbon fixation in wetlands is strongly coupled to methane production and emission to the atmosphere, with the latter representing roughly 3% of the net daily ecosystem uptake of CO₂ on a molar basis (Whiting & Chanton, 1993).

Wetlands can be thus considered as a greenhouse gas (GHG) sink since CO₂ is removed from the atmosphere and stored in the soil as carbon. On the other hand, as the CH₄ emission makes the wetlands a carbon source to the atmosphere, it is important to assess the ratio of CH₄ emitted with respect to the CO₂ sequestered (mol/mol) (Gorham, 1991; Whiting & Chanton, 2001). The imbalance in carbon fixation due to changes in primary production and microbial activities can, in fact, induce variations in the global radiative budget. Those variations are defined with the term *radiative forcing*, which denotes “an externally imposed perturbation in the radiative energy budget of the Earth's climate system” (Ramaswamy et

al., 2001) and it is expressed as $W\ m^{-2}\ p.p.m.^{-1}$. Perturbations deriving from a net increase in GHG efflux from ecosystems constitute a radiative forcing that will impact climate change and cause the increase of air temperature values. It is important to remark that each given mass of a trace gas contributes differently to the global warming, and that every greenhouse gas is described by his own *global warming potential* (GWP), which is a relative scale that compares the considered gas to that of the same moles of CO_2 (whose GWP is by convention equal to 1, according to IPCC). A GWP is calculated over a specific time interval and depends mainly on the atmospheric lifetime of the species, on the infrared absorption capacity and on the spectral location of its absorbing wavelengths. Trace gases as carbon dioxide, methane and nitrous oxide are of ecological importance for they are emitted from freshwater wetlands, and present GWP values of 1, 25 and 298, respectively, over a 20 years time horizon (IPCC, 2001). It is therefore fundamental to evaluate the radiative forcing of each gas, other than assess the amount emitted in terms of quantities. Whiting and Chanton (2001) proposed to combine the CH_4/CO_2 ratio emitted from a wetland to the variations of the GWP of the CH_4 along the time horizon considered (Figure 1.1). This is a simple method to combine the GWP curve of methane with the values obtained, for instance, from measurements or estimations of gas fluxes.

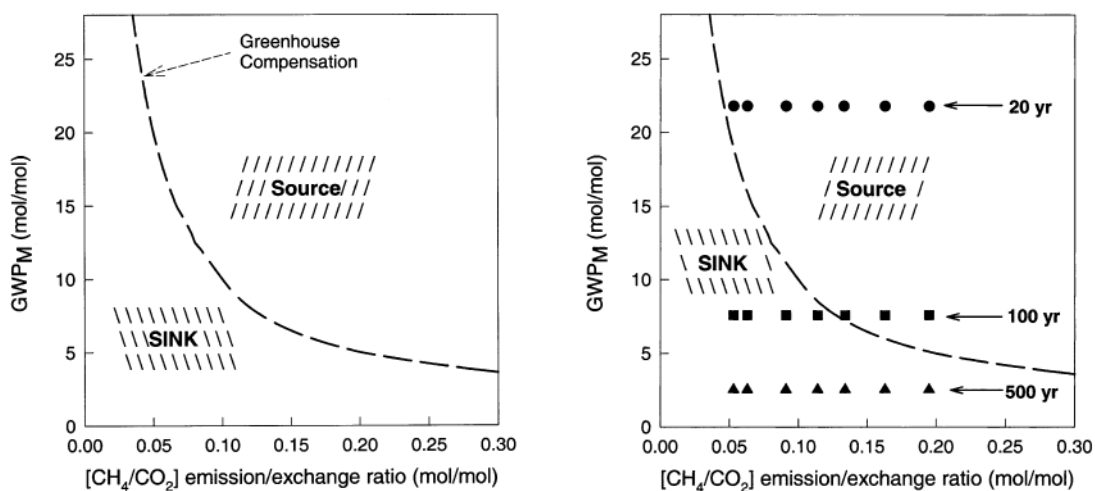


Figure 1.1. Graphical method proposed by Whiting & Chanton (2001) to evaluate whether a wetland is a source or a sink of greenhouse gases. The calculated CH_4/CO_2 ratio (mol/mol), is compared with the GWP curve of methane, which decreases with the time passing. For instance, if the ratio is >0.05 , within 20 years the studied site will be considered a source; with the same value it could be considered a sink, if the time horizon considered is longer (100 or 500 years).

1.2 Regime shift and stable states

The ecological theory of regime shifts states that a dynamic behaviour is constituted by two alternative stable states, so that continuous variations in a control variable can produce discontinuous effects. This means that most often abrupt changes in the ecosystem features are determined by responses of biotic-dependent variables to abiotic control variables or stressors (Holling, 1973; May, 1977; Collie et al., 2004). The strength of the shift of the dependent variable is determined by the nature of the relationship with the stressor: when the relation is linear, the transition is smooth and the system evolves through a continuum through steps of similar magnitude. On the contrary, in a non-linear relationship, the increased strength of the stressor can determine sudden changes in the system. This condition results in an amplified influence of the control variable and leads to a shift from a stable equilibrium to another stable equilibrium. The final state of the succession will differ in properties from the initial one and the recovery will be not possible without the supply of external energy. The shift between stable states is hysteretic, that is, the disturbances that determine the change in one direction do not have similar impacts in the opposite direction (Scheffer et al., 1993; Gunderson, 2000; Collie et al., 2004; Scheffer et al., 2001).

The theory of the regime shift is well exemplified by the changes that both freshwater and saltwater ecosystems are undergoing due to the increasing input of organic and inorganic nutrients loads and thus eutrophication (Nixon, 1995). Many authors report indeed that most shallow freshwater environments present an increase of respiration rates due to organic carbon inputs from adjacent basins or to natural evolution. The shift from an autotrophic metabolism to an heterotrophic one brings these ecosystems to be net sources of CO₂ and CH₄ to the atmosphere (Cole et al., 1994; Cole & Caraco 2001; Duarte & Prairie 2005; Bolpagni et al., 2007). In freshwater bodies, the oligotrophic-pristine and the eutrophic-degraded conditions have been assumed to represent two alternative stable states or attractors (Harlin, 1995; Scheffer et al., 2001). In this case, the primary producers community can be seen as a dependent variable, whereas the trophic level can be seen as the abiotic variable or stressor. Oligotrophic systems are characterised by high transparency and light penetration to the bottom, good oxygenation of the water column and the sediment. In those environments, the vegetation community is typically dominated by submerged aquatic vegetation (SAV), which maintains nutrient limited conditions in the water column and sediment and thus hinder excessive phytoplankton proliferation. At the same time the oxygen transport by the roots of the plant to the rhizosphere (radial oxygen loss, ROL) determine the prevalence of

aerobic microbial processes such as nitrification coupled denitrification and the retention of phosphorous bind to iron hydroxides (Jaynes & Carpenter, 1986; Neubauer et al., 2005). Natural and anthropogenic nutrient inputs (especially P and N loadings) and organic matter accumulation in sediments can generate phytoplankton abundance increase (Moss, 1976; Phillips et al., 1978; Kenney et al., 2002). In shallow lakes, phytoplankton is indeed described as the alternative state to submerged macrophyte communities (Scheffer et al., 1993; Scheffer, 2001) (Figure 1.2), as this can decrease water transparency, which determines the progressive disappearance of rooted phanerogams. Phytoplankton-dominated systems are thus characterised by elevated diurnal fluctuations in oxygen and carbon dioxide concentration in water column, mobilization of nutrients and enhanced input of labile organic matter to surface sediments.

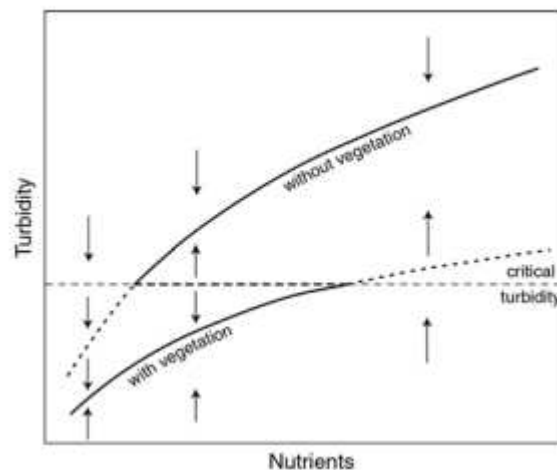


Figure 1.2. Alternative equilibrium turbidities caused by disappearance of submerged vegetation when a critical turbidity is exceeded (from Scheffer, 2001).

In a nutrient-rich system, competition among macrophyte communities can switch from vertical to horizontal pattern (Sand-Jensen & Søndergaard, 1981; Portielje & Roijackers, 1995), i.e. competition for nutrients is erased, and light fruition mechanisms become the main tool to stand out in colonising the system. In this context, floating-leaved and free-floating macrophytes are favoured because of horizontal expansion of photosynthetic organs and carbon dioxide uptake directly from the atmosphere. In brackish and coastal saltwater environments rooted plants are generally replaced by phytoplankton or floating macroalgal mats whereas in freshwater environments pleustonic or floating-leaved communities represent a stable state (Portielje & Roijackers, 1995; Scheffer et al., 2003; Viaroli et al., 2008). Pleustonic communities such as those formed by water hyacinth, water chestnut,

aquatic ferns and duckweeds, tend to establish in eutrophic systems and to support the beginning of extreme conditions in sediment and water column. The physical barrier of the floating stands limits the oxygen diffusion from the atmosphere, and contribute to maintain low levels of dissolved oxygen (DO) in water (Parr & Mason, 2004; Goodwin et al., 2008). The shading generated by high leaf density can exclude up to 99% of the incident light, thus limiting or suppressing the growth of other primary producers underwater (submerged macrophytes, green algae or phytoplankton) (Giorgi et al., 1988; Jaynes et al., 1996; Parr et al., 2002; Larson, 2007). Scheffer et al. (2003) demonstrated with a model that the prolonged colonization of the water surface by free-floating plants leads to an alternative stable state where the SAV is excluded, and that the recovery of the system can only occur at different conditions from the initial ones (Figure 1.3).

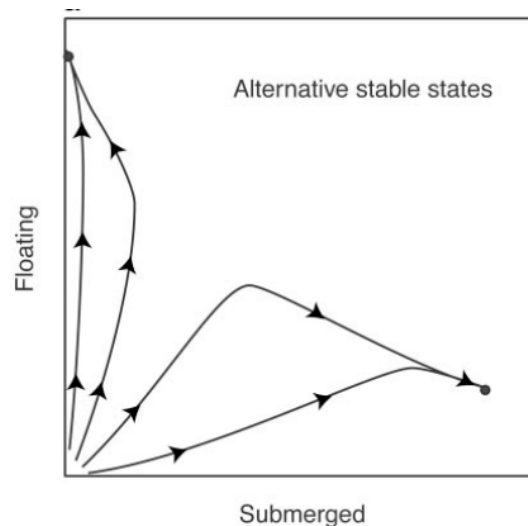


Figure 1.3. Graph taken from Scheffer et al. (2003): at low-nutrient concentrations, the only stable state is a stable state dominated by submerged plants. With increasing nutrient level, a monoculture of floating plants appears as an alternative equilibrium.

1.3 Ecology of submerged macrophyte communities

1.3.1 Oxygen transport by roots

Aquatic rooted macrophytes present a lacunar system formed by a network of intercellular gas spaces, aimed for gas transport through the plant body (Sculthorpe, 1967, Schuette, 1996). This aerenchymous tissue ensures adequate oxygen supply to the roots through diffusive transport (Sand-Jensen et al., 1982; Sorrell & Dromgoole, 1988; Schuette et al., 1994; Laskov et al., 2006). The knowledge of the oxygen transfer capacity by macrophytes is an important ecological information within the shallow aquatic environments. Radial oxygen loss (ROL) is generally expressed as the percentage of the total oxygen photosynthetically produced transferred to the rhizosphere. During the darkness, oxygen can also diffuse from the water column to the roots (Laskov et al., 2006). The fraction of the transferred oxygen varies widely, from 100% measured in isoetid species (Sand-Jensen et al., 1982) to less than 5% measured in marine phanerogams (Ottosen et al., 1999). As a rule, emergent or floating-leaved plants have higher oxygen transport capacity than submerged forms (Dacey, 1980; Armstrong and Armstrong, 1991). ROL measurements are usually performed *ex situ*, on macrophytes transplanted in agar solutions or into artificial substrates, and it is evaluated by measuring the variations of redox processes or by following the variation of oxygen leakage by means of microelectrodes (for a complete review see Colmer, 2003 and references therein). Mechanistic approaches allow to accurately measure the oxygen amount transferred to belowground organs, even if these are potential estimates, as they are obtained on roots free from sediments and therefore far from the *in situ* conditions. The real extent of oxygen loss should be biased by the sediment chemical and biological features that could influence the oxygen production by the plant and its ROL. This should be of great concern when estimating primary production with oxygen based methods. In fact, the greater is the fraction of photosynthetically produced oxygen transferred to the rhizosphere, the higher is the underestimation of primary production (Kemp et al., 1986; Pinaridi et al., 2009).

1.3.2 Effects of sedimentary organic enrichment on macrophytes

Radial oxygen loss has the potential to alter the chemical environment within sediments, with cascading effects on nutrient and gas fluxes at the water-sediment interface (Roden and Wetzel, 1996; Ottosen et al, 1999; Wigand et al., 1997, 2000). Significant effects of radial oxygen loss were demonstrated for a number of microbial and chemical reactions that alter porewater chemistry, in particular for plants growing in oligotrophic systems (Jaynes and

Carpenter, 1986; Risgaard-Petersen and Jensen, 1997), whereas its effects in organic-rich sediments are scantily explored. This should be a major concern, as eutrophication and increasing water temperature result in higher organic input to the benthic system, which forces macrophytes to improve belowground oxygen transport to counteract more reducing conditions. ROL should have profound biogeochemical consequences in organic loaded sediments as it creates oxic niches in an otherwise strictly anaerobic environment, resulting in the establishment of strong gradients between multiple oxidation and reduction zones. Sorrell et al. (2002) already found an increase in metanotrophic activity associated to roots and stems of submerged freshwater macrophytes from oligotrophic to eutrophic habitats. Wijck et al. (1992) reported that the biomass production of *P. pectinatus* was found to increase with increasing sediment organic matter content up to 26 mg C g⁻¹; with higher OM content negative effects were recorded on biomass production presumably due to highly reduced conditions and to the presence of Fe²⁺ and S²⁻ in the interstitial water. Sand-Jensen et al. (2005, 2008) investigated the effects of increasing organic matter on ROL in the isoetid species *Lobelia dortmanna*, which typically colonizes organic matter poor sediments characterized by low oxygen demand. This macrophyte loses progressively its oxygen release capacity as sedimentary OM increases; total inhibition of ROL and significant reduction of root biomass is demonstrated at relatively low OM values (2-3%) (Figure 1.4). In an analogous study, Barko and Smart (1986) investigated the effect of organic enrichment on *Myriophyllum spicatum* and *Hydrilla verticillata* growth and biomass. They found a negative correlation between organic matter and growth rates, that was explained in terms of inadequate oxygen supply to the rhizosphere; this hypothesis was anyway not verified. At high sedimentary organic matter content, mineralization rates can saturate the plant uptake capacity, resulting in pore water nutrient accumulation. Furthermore, elevated chemical and microbial oxygen consumption in organic-rich sediments minimizes the thickness of oxic layers around roots and attenuates the effects of plants on sedimentary redox conditions, and metals and phosphorous precipitation (Jaynes & Carpenter, 1986). Furthermore, when nutrient concentrations exceed certain thresholds in the water column, assimilation from the canopy is favoured keeping uptake from pore water low (Barko et al., 1991; Xie et al., 2005). On the whole, a high root:shoot ratio (RSR) is associated to plants growing in oligotrophic systems, whereas relatively higher allocation to aboveground biomass occurs in eutrophic environments; here roots mainly anchor the plants to the substrate (Denny, 1972; Barko and Smart, 1986; Van et al., 1999; Madsen and Cedergreen, 2002; Xie et al., 2005; Wang and Yu, 2007). Temporal fluctuations of the RSR, reported for *Vallisneria* spp., may reflect an

adaptive response to changing sedimentary oxygen demand within a reduced sediment (Hauxwell et al., 2007; Pinardi et al., 2009).

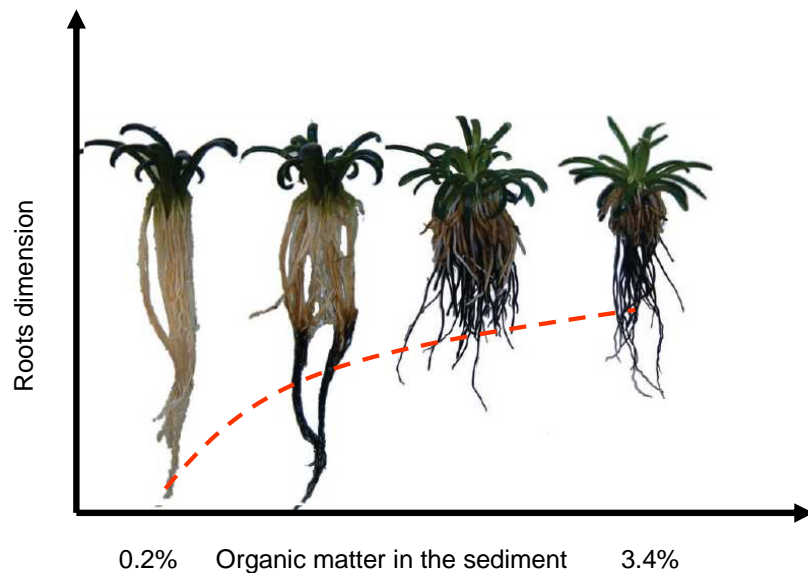


Figure 1.4. Study performed by Sand-Jensen et al. (2008) on *Lobelia dortmanna*, a submerged rooted macrophyte that sensibly suffers under even slight increases of organic matter content in the sediment.

1.3.3 Effects of submerged macrophytes on sedimentary microbial processes

Roots of submerged macrophytes can deeply affect the microenvironment within the surface and deep sediments they colonize, as a consequence of direct, i.e. solute uptake, and indirect effects, i.e. radial oxygen loss (Carpenter et al., 1983; Karjalainen et al., 2001). Submerged macrophytes rely primarily on sediments for assimilation, since the available nutrient concentration is generally much higher in pore water than in the water column (Barko et al., 1983; Carr & Chambers, 1998). Assimilation by plants has the potential to deplete sedimentary ammonium and reactive phosphorus pools, attenuating their recycling to the water column and their availability to other primary producers (Wigand et al., 2000). Furthermore, the presence of roots generally enhances the iron bound, solid-phase pool of PO_4^{3-} , augmenting the P retention capacity of the sediment (Wigand et al., 1997; Hupfer & Dollan, 2003). ROL promotes oxic conditions around roots and influences several redox-sensitive biogeochemical processes as nitrification, denitrification, iron and manganese oxidation and methanotrophy (Risgaard-Petersen & Jensen, 1997; Wigand et al., 1997; Heilman & Carlton, 2001; Sorrell et al., 2002). The canopy of macrophytes can, however, favour the local reduction of water flow, enhance sedimentation rates and retain suspended

particles, resulting in increased demand of electron acceptors within sediments (Sand-Jensen, 1998). Moreover, live roots can exude labile organic compounds while decaying roots, stems and leaves are a source of both labile and refractory organic matter (Karjalainen et al., 2001).

1.4 Ecology of floating-leaved macrophyte communities

1.4.1 Air flow mechanisms

Floating-leaved macrophytes possess thick submerged rhizomes within the sediment. This kind of plants faces the sedimentary hypoxia by conveying oxygen directly from the air to the rhizome through the aerenchyma of the petioles. The aerenchyma is formed by the web of intercellular spaces inside which air can pass continuously and transfer oxygen from leaves to hypogean parts; this structure is well-constructed especially in floating-leaved and emergent rhizophytes and in helophytes (Seago *et al.*, 2005; Jung *et al.*, 2008).

The first investigations on the air flow through the aerenchyma date back to 1850s. In 1841, two French botanists, Raffeneau-Delile and Dutrochet, compared their observations on the bubble emissions from leaves of *Nelumbo* sp. and *Nuphar lutea* (i.e. *luteum*). This debating lead the way to the following studies by Merget (1874), Barthélemy (1874) and Ohno (1910). All those studies focused firstly on the investigation of the intracellular spaces in leaves and petioles; secondly, on the influence of the environmental factors on the circulation of air; finally, on the gas composition in the inflow and outflow, respectively, especially in terms of oxygen concentration. One of the most interesting outcome was that the difference in air temperature and humidity between the inner and the outer part of the aerenchyma was the main factor in regulating the flow inside the plant.

From 1979, John Dacey evidenced that the leaves of *Nuphar lutea* present an inflow system located on the younger leaves and an outflow system on the older ones. He first understood the relevance of this system, which allow to convey oxygen to the rhizomes, to assimilate carbon dioxide directly from the atmosphere, and to indirectly release gaseous compounds deriving from plant respiration and diffusion from the sediment into the rhizome, as CO₂ and CH₄ (Dacey, 1979, 1980, 1981; Dacey & Klug, 1979, 1982). Thanks to Dacey's observations and those reported successively by Mevi-Schutz and Große (1988a, 1988b), two main gas transport patterns were proposed. In the *Nelumbonaceae*, gas exchange with atmosphere takes place on the same leaf: air inflow and outflow are located on the rim and on the central part of the leaf, respectively, and take advantage from a two-way transport structure within the petiole (Dacey, 1987; Mevi-Schutz & Große, 1988a) (Figure 1.5). On the contrary, in the *Nymphaeaceae*, the inflow and the outflow are located on leaves of different age, which are originated from the same rhizome segment (Dacey, 1981) (Figure 1.6). The inverse direction of the air flow is attributable to different width of the stomata on different aged-leaves (Große & Bauch, 1991). New born leaves present indeed smaller stomata, which generate high pressure conditions. The entire plant shows an high inner pressurization, which cannot in turn maintained by the older leaves, that present broader stomata. Those conditions thus

generate an air inflow through the younger leaves, and the air leakage through the petioles and the blades of oldest leaves. The convective motion involves various environmental compartments (atmosphere → plant, as young leaves → plant, as rhizome → plant, as old leaves → atmosphere) and results in a elevated air flux. In 1981, Dacey measured an air flow of 50 ml min⁻¹ moving inside petioles of *N. lutea*; investigations on other plants report fluxes comprised between 10 and 80 ml min⁻¹ (for a complete review, see Große, 1996).

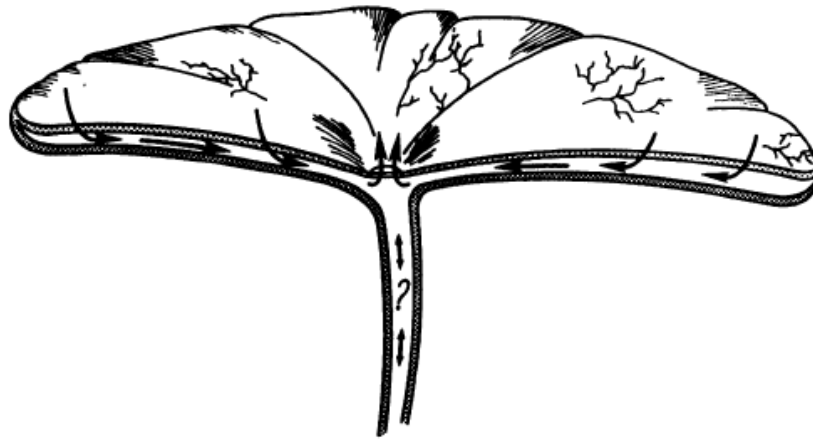


Figure 1.5. Scheme of the two-way transport through the leaves of *Nelumbo* sp. according to Dacey (1987). The air enters into the smaller pores on the rim of the blade and comes out from the central channel of the petiole. The two-way transport will be further investigated by Mevi-Schutz & Große (1988a).

In one of his first studies in 1981, Dacey tried to understand which was the mechanism triggering the air flow through the petioles of *N. lutea*. He had already found out that a porous wall was inside the leaf, and that it was formed by the intracellular spaces with diameter <0.1 μm. This diameter does correspond to the free mean path of gas molecules, i.e. this space is hardly sufficient to let a gas molecule to pass by diffusion. Moreover, closed stomata don't reach this diameter: therefore, air flow measured at night is not explainable by diffusion mechanisms (Dacey, 1980; Dacey & Klug, 1982; Schröder *et al.*, 1986; Dacey, 1987). There must be some other mechanism other than diffusion in regulating the air flow. Finally, Große & Bauch (1991) and Große (1996a, 1996b) resumed the mechanisms involved in the process of the mass flow. In plants, thermo-osmotically active partitions are present inside leaves and petioles of floating-leaved and emergent rhizophytes: those partitions are regulated by pressurization generated from temperature and humidity variations. Those structures are formed both by a porous wall, which determines the molecular flow through intercellular spaces (called thermal molecular flow, according to Knudsen, 1910) and by a

non-porous membrane, which determines the gas solubility within the cytoplasm and cellular walls (called thermo-osmosis, according to Denbigh & Raumann, 1952). Moreover, during isothermal conditions, i.e. transition phases between extreme temperatures (early morning, late afternoon), the gas flow is regulated by pressurization caused by differences in humidity (called hygrometric pressurization, according to Dufour, 1874). From these studies, further in-depth examinations focused on other floating-leaved and emergent hydrophytes: *Nymphoides peltata* (Große & Mevi-Schutz, 1987), *Victoria amazonica* (Große et al., 1991) and *Euryale ferox* (Große & Bauch, 1991).

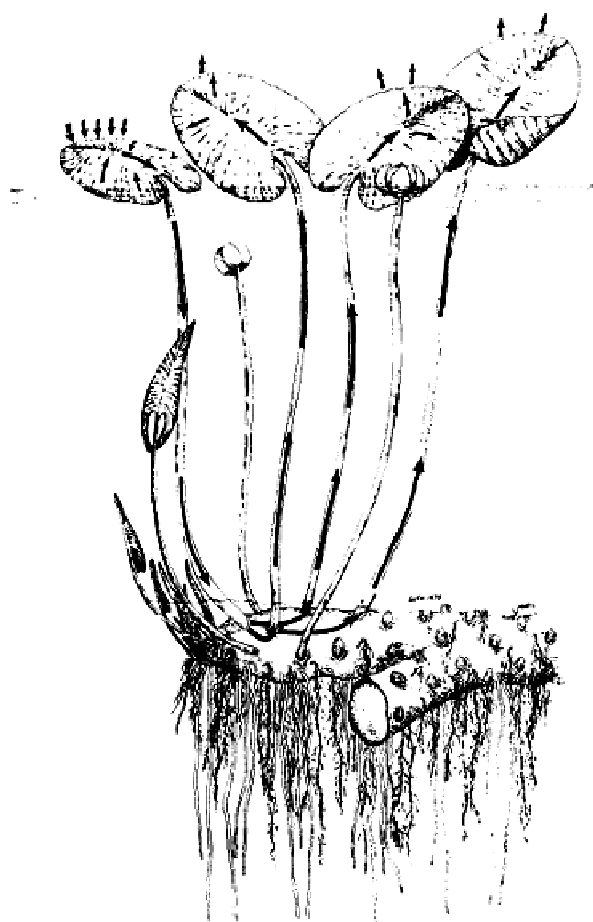


Figure 1.6. Scheme of the inner gas transport system through the leaves of *N. lutea* according to Dacey (1981). Oxygen-rich air enters stomata on the younger leaves and it is conveyed to the rhizome and to the roots. Air outflow is located on the older leaves and it is mainly composed by carbon dioxide and methane.

1.4.2 Ecological relevance of the mass flow inside aerenchyma

Since its first investigations, Dacey put in evidence the relevance of the gas composition in the air inflow (mainly O₂ and CO₂) and in the outflow (mainly CO₂ and CH₄) (Dacey & Klug, 1979). Oxygen transport is indeed fundamental for roots and rhizome survival;

moreover, oxygen leakage creates oxic micro-niches around roots, which favour aerobic microbial processes. Aerenchyma-mediated transfer is one of the processes that convey methane from the sediment to the atmosphere, other than molecular diffusion in water and bubbles escape (Schutz *et al.*, 1991) (Figure 1.7); the predominance of one process depends on the turbulence conditions in water, from the organic load in the sediment and from the typology of primary producers community. In conditions of low water turbulence, the sedimentary oxic layer is restricted to the first horizon (0-3 cm): methane produced in the deeper layers accumulates in bubbles which escape in water column and then to the atmosphere (Chanton *et al.*, 1989).

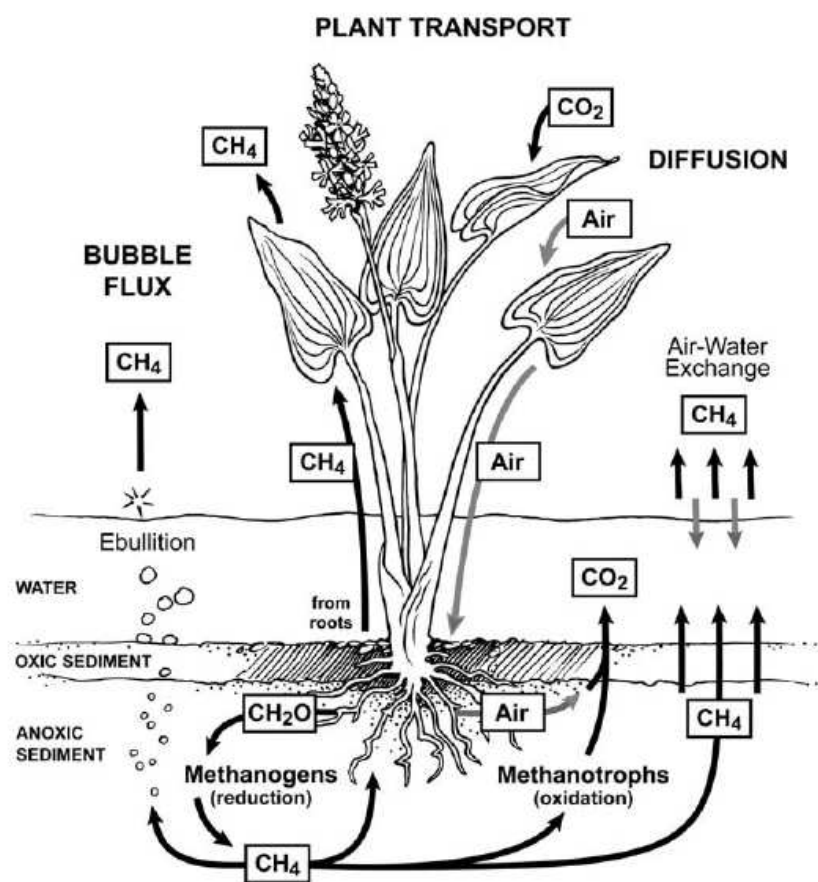


Figure 1.7. Scheme of methane production, re-oxidation and escape at the sediment-water-air interface, according to Schutz *et al.* (1991).

In shallow lentic freshwaters characterized by a high OM content (ponds, oxbow lakes, peats, marshes) the occurrence of floating-leaved and emergent rhizophytes is the main factor regulating the methane efflux towards the atmosphere. In fact, oxygen conveyed to the rhizosphere through the aerenchyma significantly contributes to methanotrophy; in the same way, methane produced within the sediment can diffuse into the rhizome without being

reoxidised and be transferred directly to the atmosphere by the ventilation inside the aerenchyma (Colmer, 2003). The relative responsibility in terms of emissions of the riparian vegetation is by now confirmed: different authors report that fluxes measured on littoral vegetated areas are sensibly higher when compared to those from open waters (Smith & Lewis, 1992; Juutinen *et al.*, 2003; Kankaala *et al.*, 2003; Wang *et al.*, 2006). Many studies have already been made about methane emissions from helophytes (van der Nat & Middelburg, 1998; Brix *et al.*, 2001; Käki *et al.*, 2001; Kankaala *et al.*, 2005), while a few investigations still exist about floating-leaved macrophytes. Among them, Smith & Lewis (1992) measured methane efflux from *N. lutea* by means of a floating chamber and reported fluxes of about 12.6 mmol m⁻²d⁻¹, being that measured on free water 4-fold lower. Of the same order of magnitude are fluxes reported about the same species by Juutinen *et al.* (2003), of 0.4 mol m⁻² ice free period⁻¹, and by Kankaala *et al.* (2003), of 10 mmol m⁻²d⁻¹; in those cases too, fluxes from leaves were significantly higher than those attributable to solely diffusive processes in open waters.

1.5 Ecology of free-floating macrophyte communities

1.5.1 Physiology of free-floating plants

Free-floating plants (FFP), also cited as floating aquatic weeds (FAWs) or pleustophytes, encompass a large number of species which are characterized by floating leaves and small or reduced roots, mostly free in the water column (duckweeds and aquatic ferns), or faintly rooted into the sediment (water hyacinth, water lettuce and water chestnut). Pleustophytes are characteristic of eutrophic waters, both flowing and stagnant: the great nutrients availability allows these plant communities to grow far from the surface sediment, since most of the nutrient uptake occurs from the inferior side of the leaves (Filbin & Hough, 1985; De Groot et al., 1987; Portielje & Roijackers, 1995; Boedeltje et al., 2005). Moreover, floating leaves allow to take advantage of direct light, by avoiding turbidity in water column due to high dissolved organic matter. In eutrophic freshwater environments, pleustonic communities can establish as a stable state as they promote their self maintenance (Portielje & Roijackers, 1995; Scheffer et al., 2003), thanks to high growth rates (up to 0.3 d⁻¹, Hillman, 1961; Driever et al., 2005) and both sexual and asexual reproduction (Howard & Harley, 1998; Boedeltje et al., 2004, 2005).

1.5.2 Ecological impacts of free-floating plants invasion

Pleustophytes are known for infesting worldwide both flowing and stagnant waters, with dramatic negative effects on water oxygenation (Morris & Barker, 1977; Pokorný & Rejmánková, 1983; Caraco & Cole, 2002; Parr & Mason, 2004; Bolpagni et al., 2007), limitation of autotrophy and enhancement of heterotrophs community (Cattaneo et al., 1998; Fontanarrosa et al., 2010), decrease of plankton abundance and richness (O'Farrell et al., 2009), suppression of submersed macrophytes growth (Sculthorpe, 1967; Portielje & Roijackers, 1995; Parr et al., 2002) and generalized damages on fishing industry and boats viability (Charuttadan 2001; Williams & Hecky, 2005). The effects of their presence are strictly connected to the percentage of water covered, so that the worst negative effects are mostly remarkable during late summer, when plants reach the maximum standing crop (Meerhoff et al., 2003; Pierobon et al., 2010). With increasing water temperature, the growth rate increases approximately linearly up to an optimum (Landolt, 1986; van der Heide et al., 2006).

The larger species present the most potential threat for the environment, as they can reach elevated biomass (up to 600 g_{DW} m⁻², Bolpagni et al., 2007) and colonize the whole water surface. The most challenging species have a pan-tropical distribution: *Eicchornia crassipes*

(water hyacinth) which is typically present in Africa (Charuttadan 2001; Williams & Hecky, 2005); *Salvinia molesta* (salvinia fern) whose infestations are mainly reported for Sri Lanka (Doeleman, 1989) and Kenya (Njuguna, 1992); *Pistia stratiotes* (water lettuce) mainly diffused in Australia (Harley et al., 1990). *Trapa natans* (water chestnut) is typical of the boreal hemisphere and is reported in the U.S.A. (Caraco & Cole, 2002), Europe (Cattaneo et al., 1998; Bolpagni et al., 2007) and Japan (Tsuchiya, 1983). Smaller species, such as *Lemnaceae*, are diffused mostly in stagnant shallow waters and in ditches of the boreal hemisphere (Pokorný & Rejmánková, 1983; Boedeltje et al., 2005; Buczkó, 2007) and can reach standing crop of more than 500 g_{DW} m⁻² and density of 12,000 turions m⁻² (Boedeltje et al., 2005) (Figure 1.8).

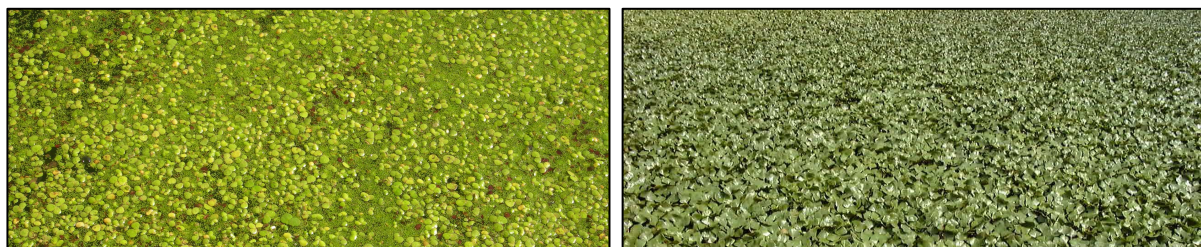


Figure 1.8. During their maximum standing crop, free-floating plants cover the whole surface of the basin they colonize. On the right, duckweeds covering (*Lemna gibba* and *minuta*); on the left, aquatic ferns covering (*Salvinia natans*) in two ponds of the Northern Italy.

The effects of oxygen depletion in water column due to pleustophyte spreading are easily detectable in low redox potential (E_h) values, with cascading effects on nutrients availability, such as NH_4^+ and PO_4^{3-} . Nutrients mobilization due to anoxia generates a positive feedback which maintains a high nutrient level in water and sediment, assuring the ideal conditions for the mat establishment, which is kept stable by seed and propagule banks within the sediment (Boedeltje et al., 2004, 2005; Bolpagni et al., 2007). Even though oxygen depletion is by now an evidence in environments colonized by pleustophytes, little has been done to investigate the effect of those plants on methane emissions (Bolpagni et al., 2007; Pierobon et al., 2010). The prolonged colonization of the water surface by this plant typology along several months during the year generates an oxygen deficit in water column, which makes anaerobic processes to prevail over the aerobic ones with consequent production of reducing compounds. In those conditions, the reoxidation of methane produced in sediments would be likely negligible compared to that of environments colonised by rooted macrophytes, being also an important amount of the carbon fixed regenerated toward the atmosphere as methane.

2. Problem statement

2.1 Topic context

Recent studies evidence that nutrients and organic matter input in freshwaters determines a community shift in primary producer communities, with cascade implications on the intensity and direction of benthic processes. The main effects of this change are related to water oxygenation, and thus to the strength of the microbial respiration and reoxidation processes at sediment-water-air interfaces. Basically, every type of hydrophytes present structural adaptations to face anoxic sediments and to take advantages of nutrient availability in water column. In an increasing nutrient enrichment gradient, pleustophytes and floating-leaved macrophytes tend to replace submerged macrophytes communities, as they uptake nutrients directly from the water and oxygen from the air. Those primary producers lead the system toward a stable state that shuts out other communities and sustains their self-maintenance; the first consequence are the oxygen depletion in water and the increase of anaerobic processes. Freshwater environments dominated by different primary producers will thus have dissimilar characteristics in terms of water oxygenation, methanogenesis, methanotrophy and efflux of GHG towards the atmosphere.

2.2 Objectives and structure of the thesis

The overall aim of my work thesis is to put in evidence the role of different primary producers (specifically hydrophytes) in regulating the water and sediment oxygenation in shallow wetlands and thus the consequences on benthic processes, with particular regards to the carbon budget and methane emissions towards the atmosphere. The approach I used is meant to obtain both general and particular views of the problem, in order to emphasize some aspects which have not yet been taken into account in previous studies.

The first part of the work (Chapter 5 and 6) concerns the measurements of the benthic processes at the sediment-water interface in an environment dominated by a submerged rooted macrophyte (*Vallisneria spiralis*). This part aims at delving into the dynamics of oxygen transport in the sediment by submerged hydrophytes, which are known to considerably contribute to the oxidation processes and the maintenance of good water

quality. Measurements of methane and carbon dioxide fluxes were performed in sediments colonized by rooted submerged macrophytes under controlled conditions in a mesocosm experiment. Secondly, the short-term effects of the colonization of this macrophyte were investigated at two sites differing for the organic matter content in sediments.

The basic questions of the experiments were:

- Which are the short-term effects of the colonization of an organic sediment by a rooted submerged macrophyte on pore water chemistry?
- Which are the effects of the presence of a rooted submerged macrophyte on O₂, CO₂ and CH₄ fluxes across the sediment-water interface along an organic gradient?
- Is the oxygen transport to the rhizosphere impacted by different degree of organic matter content?

The second part of the work (Chapter 7) is dedicated to measure the carbon dioxide and methane emissions at the water-air interface of a *Nuphar luteum* vegetated stand under natural conditions. Floating-leaved macrophytes are usually studied with particular concerns to the methane emissions through the aerenchyma; on the contrary, the carbon uptake by this plant is still barely considered. However, this is an indispensable piece of information in order to assess whether the plants have the net effects of fixing or releasing carbon, and thus impacting the role of a wetlands as a sink/source of GHG to the atmosphere. Secondly, scarce knowledge exists about the effects those plants determine on water chemistry: in particular, whether the floating-leaved plants induce anoxia in the water column because of the physical barrier they form.

The basic questions of the study were:

- Is the carbon fixation by the plants balancing the methane emissions through the aerenchyma in terms of carbon dioxide equivalents?
- Which is the influence of this type of hydrophyte on water chemistry, in terms of water and sediment oxygenation and dissolved methane in the water?

The third part of the work (Chapter 8) is focused on calculating theoretical fluxes of GHG in a variety of shallow aquatic environments colonized by a different hydrophytes. This activity has the main objective of evidencing the impacts of the dominance of each different plant community on the gas dynamics in water. In particular, I wanted to investigate the net effect of the growth of the primary producers along the year in a *in situ* condition, in order to follow the natural evolution of the biotic-abiotic system. There are indeed only a few experimental

evidences about the mechanisms that bring the pleustophyte-dominated sites to promote their establishing in a stable state.

The basic questions of the monitoring were:

- Is there a strong difference between sites colonized by different hydrophytes in terms of gas (O_2 , CO_2 and CH_4) saturation and of greenhouse gas emissions?
- Are the dissolved gas dynamics along the year dependent on the type of dominant hydrophyte?

3. Study area

The study area considered within this project consists of 21 shallow freshwater systems located in the Po River Plain, within the basins of the Po, Oglio and Mincio rivers, in the provinces of Cremona and Mantua, Northern Italy (Figure 3.1 and Table 3.1). Two sites are located within the Mincio River and were used only for material collection for the onset of indoor experiments. The wetlands here considered are small-sized and shallow, differ one from another in depth, surface and connection degree to the river basin (Racchetti et al., 2010) and enclose the main characteristics of the majority of wetlands in the Po Plain.

The Po River Plain is characterised by intensive agricultural practices, livestock farming and breeding (Marchetti 1993). The studied wetlands are eutrophic to hypereutrophic and are undergoing rapid infilling; most of them are included in the Oglio Nord, Oglio Sud, and Mincio Natural Parks. The primary water sources in the isolated wetlands (mainly ponds and marshes) are groundwater, precipitation and runoff, with the water movement due largely to vertical fluctuation; connected wetlands (i.e. oxbow lakes) receive water and nutrients from river flooding.

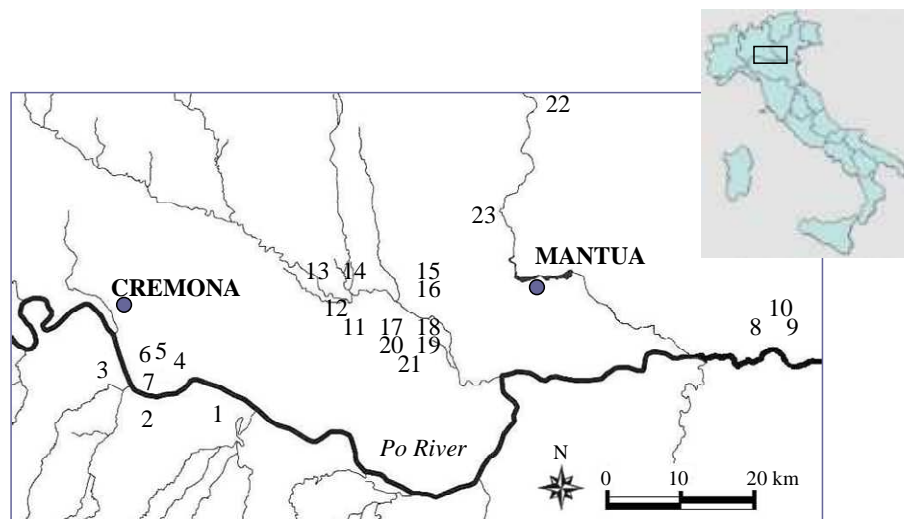


Figure 3.1. The map shows the location of the sampling sites, all within the Po River Plain.

Table 1. Main features of the investigated sites. Chemical data are taken from unpublished studies performed between 2007 and 2010 (courtesy of Elisa Soana), and from Longhi et al. (2008) and Racchetti et al. (2010). The sites are dominated by different aquatic vegetation, indicated as SM = Submerged macrophytes, PH = Phytoplankton, FL = Floating-leaved macrophytes, PL = Pleustophytes. Plant species were identified by Dr. Rossano Bolpagni.

Name	Wetland typology	Area (ha)	Depth range (m)	Aquatic Vegetation	Dominant Species	[PO ₄ ³⁻] (μM)	[NH ₄ ⁺]/[NO ₂ ⁻ +NO ₃ ⁻]	OM (%)
Ragazzola	Pond (old river reach)	0.20	1 - 4	PL	<i>Lemna gibba</i> / <i>Lemna minor</i>	4.6	2.3	9
Ongina morta	Oxbow lake	5.10	1 - 1.5	FL	<i>Nuphar luteum</i>	0.9	0.6	6
Lancone di Po	Oxbow lake	6.19	0.5 - 2	SM / PH	<i>Ceratophyllum demersum</i> / <i>Phytoplankton</i>	0.4	0.7	12
Santa Margherita	Natural Pond	0.24	0.5 - 2	FL	<i>Nuphar luteum</i>	0.6	11.2	14
Ca' de Gatti	Natural Pond	0.28	0.5 - 1.5	PL	<i>Lemna minor</i> / <i>Wolffia arrhiza</i>	1.2	7.7	27
Lazzaretto	Natural Pond	1.25	1 - 2	PL	<i>Lemna minor</i> / <i>Spirodela polyrhiza</i>	1.7	13.2	29
Gerre Ugolani	Natural Pond	0.39	0.5 - 1.5	SM	<i>Ceratophyllum demersum</i>	0.4	5.2	7
Busatello 1	Freshwater Marsh		1.5 - 2	SM	<i>Ceratophyllum demersum</i>	0.3	0.5	17
Busatello 2	Freshwater Marsh	10.50	1.5 - 2	PH	<i>Phytoplankton</i>	0.3	0.1	24
Busatello 3	Freshwater Marsh		1.5 - 2	FL	<i>Nuphar luteum</i> - <i>Nymphaea alba</i>	0.2	0.2	26
Castelfranco	Oxbow lake	0.20	0 - 0.5	PL	<i>Salvinia natans</i>	0.1	50.6	27
Bicocca	Oxbow lake	0.29	0.1 - 0.5	PL	<i>Lemna gibba</i> / <i>Spirodela polyrhiza</i>	8.1	18.7	22
Runate destra	Oxbow lake	0.18	1 - 2	FL	<i>Nuphar luteum</i>	0.3	0.0	3
Runate sinistra	Oxbow lake	0.29	1 - 2	FL	<i>Nuphar luteum</i>	0.3	0.0	13
Pesca destra	Pond (old peat bog)	0.25	1 - 2	FL	<i>Nuphar luteum</i>	0.2	0.0	14
Pesca sinistra	Pond (old peat bog)	0.27	1 - 2	FL	<i>Nuphar luteum</i>	0.2	0.1	15
Le Bine	Oxbow lake	12.08	0.1 - 0.5	PH	<i>Phytoplankton</i>	0.9	14.1	16
Belforte 1	Marsh (old peat bog)	0.39	0.1 - 0.4	PH	<i>Phytoplankton</i>	0.2	5.4	18
Belforte 2	Pond (old peat bog)	0.20	1.5 - 2	FL	<i>Nymphaea alba</i>	0.2	7.2	21
Belforte 3	Pond (old peat bog)	0.10	1.5 - 2	FL	<i>Nuphar luteum</i>	0.2	10.1	17
Marcaria	Freshwater Marsh (old peat bog)	1.09	1 - 1.5	PH	<i>Phytoplankton</i>	5.1	1.5	34
Peschiera	River		5 - 6	SM	<i>Vallisneria spiralis</i>	0.3	0.4	6
Massimbona	River		1.5 - 2.5	SM	<i>Vallisneria spiralis</i>	1.7	0.1	10

4. Methods

4.1 Water sampling and related analyses

4.1.1 Collection and treatment of the sample

When collected *in situ*, water was sampled with a plastic bottle (1 L) and then transferred into a glass vial (Exetainer Labco, High Wycombe, UK; volume 12 ml), where Winkler reagents were immediately added for later titration of dissolved oxygen (O_2) (Strickland & Parsons 1972). In the meanwhile, water temperature ($^{\circ}C$) and pH were recorded by means of a YSI Multiple Probe (mod 556). An aliquot was filtered and transferred to a glass vial for later titration of dissolved inorganic carbon (DIC). Samples for nitrate (NO_3^-) analyses were filtered and transferred into 50 ml plastic vials. A variable amount of water (100-500 mL) was filtered with a Whatman GF/F filter and put in a plastic vial for chlorophyll-*a* (Chl-*a*) analysis. All vials were put into a refrigerated box and brought to the laboratory. When collected in laboratory, water was usually sampled by means of plastic syringe (variable amount depending on the experiment, see details throughout the text) and treated as described above.

4.1.2 Titration

Dissolved oxygen (O_2) was determined with Winkler titration (A.P.H.A., 1981), while dissolved inorganic carbon (DIC) was determined with acidimetric titration with HCL 0.1 N (Anderson et al., 1986). Dissolved carbon dioxide (CO_2) was calculated from pH and DIC values according to the formula described in Lewis & Wallace (1998).

4.1.3 Spectrophotometric analyses

Nitrate (NO_3^-) was measured after reduction to NO_2^- with activated cadmium (Golterman et al., 1978). Chlorophyll-*a* (Chl-*a*) concentration was determined spectrophotometrically after 24h-extraction with 90% acetone (Lorenzen 1967).

4.2 Air sampling and related analyses

4.2.1 Collection and treatment of the sample

For the analyses of dissolved gases (CO₂ and CH₄) in water, gas samples were obtained through the headspace equilibration (McAuliffe, 1971; Cole et al., 1994) by transferring a 600 ml water sample into a glass heavy-walled dark bottle (1100 ml) equipped with a gas-tight septum. The bottle was immediately closed and vigorously shaken for 60 seconds; gas samples were collected from the headspace by means of plastic syringes (30 ml) and carefully flushed into gas-tight vials (12 ml). Prior tests performed on several replicates demonstrated that the entire procedure matched with CO₂ calculation obtained from pH and DIC measurements. Gas collection from the headspace of the floating chambers was performed by means of plastic syringe (60 ml).

4.2.2 Gas chromatographic analyses

Methane (CH₄) analyses were performed with a Fisons 9000 series gas chromatograph equipped with a flame ionization detector (FID). The oven temperature was maintained at 60 °C; the temperature of injection and detector was held at 220 and 250 °C, respectively. The flow rate of helium carrier gas was 3 ml/min, as well as that of the air.

Carbon dioxide (CO₂) concentration was measured with a ThermoFinnigan gas chromatograph equipped with a thermal conductivity detector (TCD). The oven temperature was maintained at 33 °C; the temperatures of the injector and detector were each 100 °C, respectively. The flow rate of helium carrier gas was 1.5 ml/min.

4.2.3 Calculation of dissolved gas concentration in water

When collected with the headspace equilibration technique, measured gas partial pressure in the gaseous phase was converted in concentration as follows:

$$1) \quad [g] = P_g \times K_h$$

where $[g]$ is the concentration of the given gas (mol L⁻¹), P_g is the partial pressure of the given gas (atm), and K_h is the Henry's constant for the given gas at a given water temperature and salinity (mol L⁻¹atm⁻¹) (reported in Sander, 1999, and references therein). The concentration obtained this way indicated therefore the concentration of the gas in the

atmosphere or in the water, assuming that the gas had equilibrated between the aqueous and the gaseous phases according to the Henry's law. This amount was thus corrected for the original number of moles contained in the gaseous volume (i.e. the atmospheric partial pressure) and the water volume. I considered atmospheric partial pressures of 0.000314 and 0.00000179 atm for CO₂ and CH₄, respectively (IPCC, 2001).

Carbon dioxide concentration values calculated from titration and measured with the headspace technique were compared, and the relationship between the two methods was described by the following equation:

$$\text{CO}_{2,\text{tit}} = (1.011 \pm 0.028) \text{CO}_{2,\text{GC}} - (0.002 \pm 0.006)$$

The regression between the two series resulted statistically significant ($R^2=0.76$, $p<0.01$, $n=421$; see figure 4.1), indicating the reliability of both methods for *in situ* measurement. The slight overestimation with the titration method at high concentrations could be due to errors in pH measurement, as proposed by Raymond et al. (1997), who had found a much higher discrepancy between the two series of numbers.

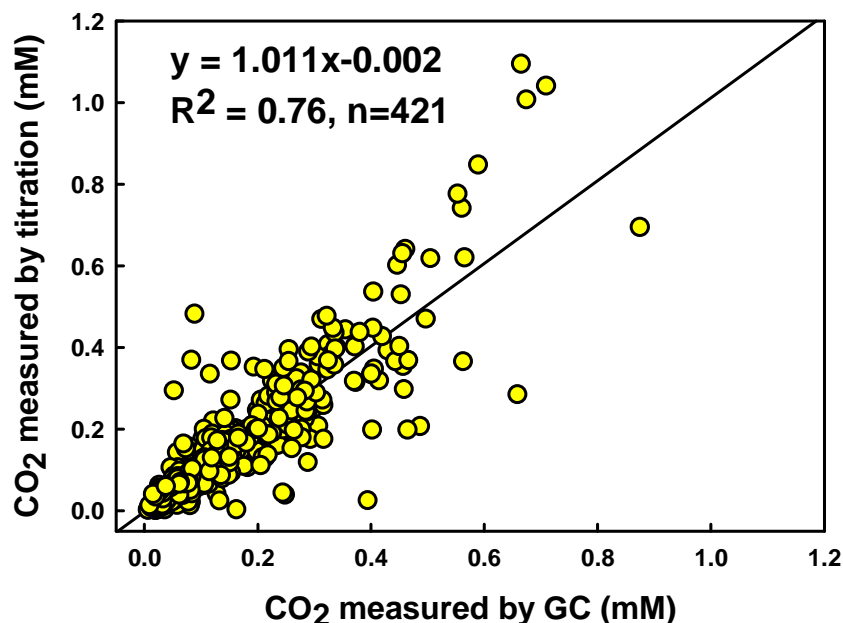


Figure 4.1. Direct (gas chromatography) and indirect (calculation from pH and DIC values) determination of CO₂ concentration in 421 water samples.

4.2.4 Calculation of dissolved gas saturation in water

Dissolved gas saturation in water was expressed as follows:

$$2) \quad \% = \frac{[g]_s}{[g]_t} \times 100$$

where the % indicates the saturation of the given gas, $[g]_s$ is the gas concentration in the sample (mM) and $[g]_t$ is the theoretical concentration of the gas in the water at the equilibrium with the atmosphere (mM). Theoretical gas concentration in water ($[g]_t$) was calculated according to the eq. 1), considering atmospheric partial pressures of 0.21, 0.000314 and 0.00000179 atm for O₂, CO₂ and CH₄, respectively (IPCC, 2001) and the water temperature at the moment of sampling.

4.3 Sediment sampling and related analyses

Sediments were collected by means of Plexiglas cores of different size according to the experiment (see details for each section). Sediment density was measured on slurries, by weighting 5 cm³ of fresh material; porosity (as ratio between water weight and fresh sediment volume) was calculated after desiccation at 50°C until constant weight. On dry, powdered sediments, organic matter content (OM, %) was quantified as loss on ignition (LOI) at 350°C for 2 hours.

5. Influence of radial oxygen loss by a submerged macrophyte (*Vallisneria spiralis*) on gas and nutrient fluxes at the sediment-water interface

5.1 Aim of the study

Radial oxygen loss has the potential to alter the chemical environment within sediments, with cascading effects on nutrient and gas fluxes at the water-sediment interface (Roden and Wetzel, 1996; Ottosen et al, 1999; Wigand et al., 1997, 2000). Significant effects of radial oxygen loss were demonstrated for a number of microbial and chemical reactions that alter porewater chemistry, in particular for plants growing in oligotrophic systems (Jaynes and Carpenter, 1986; Risgaard-Petersen and Jensen, 1997), whereas its effects in organic-rich sediments are scantily explored. This should be a major concern, as eutrophication and increasing water temperature result in higher organic input to the benthic system, which forces macrophytes to improve belowground oxygen transport to counteract more reducing conditions. ROL should have profound biogeochemical consequences in organic loaded sediments as it creates oxic niches in an otherwise strictly anaerobic environment, resulting in the establishment of strong gradients between multiple oxidation and reduction zones.

The aim of this study was to provide evidences of the elevated belowground oxygen transfer by the submerged macrophyte *Vallisneria spiralis* growing in organic-rich sediments. As *V. spiralis* successfully colonizes a wide range of substrates, from gravel to fluffy organic sediments, we hypothesize that ROL is enhanced in organic-rich sites, resulting in an unbalanced stoichiometry between TCO_2 and O_2 benthic fluxes and in CH_4 oxidation.

5.2 Sampling program and calculations

5.2.1 Microcosms setup

Plants, sediment and water were collected from the northern reach of the Mincio River, a 75 km long tributary of the Po River originating from the Lake Garda (Northern Italy). Two sampling sites were investigated, located respectively upstream (site 22) and downstream (site 23) a wastewater treatment plant. At both sites the submerged macrophyte *V. spiralis* is the most representative species within the aquatic plant community (about 90% of cover abundance). Large canopies reduce locally the water flow and enhance the accumulation of

particulate matter within the meadow. In turn, the buildup of nutrient-rich muddy substrates has a positive feedback for the plant growth and meadow expansion, which develops into dynamic patches (Madsen et al., 2001). The chosen sampling sites are characterized by soft sediments, about 20-40 cm thick, laying upon a pristine gravel bottom. Sediments at the two sites have similar density, but they differ for organic matter (6% and 10% as loss on ignition, upstream and downstream, respectively).

In March/April, July/August and October/November 2008, O₂, TCO₂ and CH₄ flux measurements were performed in microcosms containing bare and *V. spiralis* vegetated sediments collected from the upstream and downstream stations. The experimental schedule was planned to cover three key stages of the macrophyte, namely the exponential growth phase (spring), the biomass peak (summer) and the senescence phase (autumn) (Pinaridi et al., 2009). When collecting intact cores, sediment disturbance and damages to the rhizosphere are difficult to avoid, especially when considering stoloniferous species as *V. spiralis*. We therefore choose an alternative approach, based on the transplant of single individuals into microcosms with sieved sediment, followed by an acclimatization period under natural conditions. This procedure allows to reduce small-scale heterogeneity and ensure that all roots are transplanted in the same medium.

At each site about 20 L of sediment were collected by means of plexiglas liners from the upper layer (0-10 cm), sieved with a 0.2 cm mesh and homogenized. Concomitantly, about 50 *V. spiralis* shoots with intact rhizosphere were carefully collected from the meadow by scuba divers and sorted by uniform size for the transplant. Homogenized sediments were transferred into 16 cylindrical plexiglas microcosms (o.d. 8 cm, height 10 cm); in 8 of them, 2 to 3 *V. spiralis* shoots were transplanted (Figure 5.1). The number of transplanted plants was determined from the shoot density measured *in situ* (0.5 x 0.5 m plot, n=3). Hence, 8 vegetated microcosms and 8 unvegetated microcosms were realized at each site and in each sampling periods. Besides, a subsample of sieved sediment was collected for the analyses of density, porosity, water and organic matter content according to standardized methods (see chapter 4). All microcosms were then transferred within vegetated and non-vegetated patches, and left in the river bottom for 20 days under natural temperature, light and flow conditions (Figure 5.1).

Thereafter, microcosms were carefully retrieved, transferred underwater into plexiglas liners (i.d. 8 cm, height 30 cm) equipped with a rubber stopper on the bottom and submerged with the top open in tanks containing *in situ* water (Figure 5.2). River water temperature was measured with a YSI Multiple Probe (mod 556) and PAR intensity with a luxometer (Delta OHM, HD9021 model). Liners containing microcosms were then transported to the

laboratory, together with 100 L of *in situ* water. Here, all liners were equipped with a water stirring unit (a teflon coated magnetic bar) positioned in the upper portion of the liner, with aim of gently mixing the water column (40 rpm) without suspending sediments or touching the plant fronds. Within two hours from sampling, liners with vegetated and unvegetated microcosms were transferred and maintained in pre-incubation tanks as described in Dalsgaard et al. (2000) (Figure 5.2).



Figure 5.1. From left to right, the sequence of transplant of *V. spiralis* shoots.



Figure 5.2. From left to right: acclimatization on the river bottom, microcosms collection, incubation system in laboratory.

5.2.2 Dissolved oxygen, inorganic carbon and methane ecosystemic fluxes

Incubations for oxygen, inorganic carbon and methane flux measurements were performed in the light and in the dark at *in situ* temperature, according to standard protocols (Dalsgaard et al., 2000; Pinardi et al., 2009). Halogen lamps, reproducing average *in situ* irradiance, were used in light incubations. Incubation time (3 to 6 hours) was set in order to keep the variation of dissolved O₂ within 20-30% of the initial value. When incubations were started each liner

was provided with a top plexiglas lid with a water sampling port. Water temperature and irradiance during the two sets of three incubations reflected average *in situ* values, and were of 12, 24 and 17 °C and 300, 500 and 200 $\mu\text{E m}^{-2}\text{s}^{-1}$ in spring, summer and autumn, respectively.

During the incubation, water samples were collected from each liner 3 times (initial, intermediate, final) with plastic syringes and transferred into glass vials (Exetainers, Labco, High Wycombe, UK). Dissolved oxygen (O_2) and dissolved inorganic carbon (TCO_2) and dissolved methane (CH_4) were determined according to methods described in chapter 4.

Hourly fluxes of O_2 , TCO_2 and CH_4 were calculated with a linear regression of concentrations *versus* incubation time, and expressed as rates per square meter ($\text{mmol m}^{-2}\text{h}^{-1}$). Net Ecosystem Production (NEP) values refer to oxygen and inorganic carbon fluxes measured during light incubations; Ecosystem Respiration (ER) values refer instead to fluxes measured in the dark. Net Ecosystem Metabolism (NEM, Odum, 1969) refers to daily rates, obtained by multiplying hourly rates for the correspondent light and dark hours of the sampling period and then by summing them. These rates were subsequently extended to the whole sampling period (May-November 2008 for the site U, and April-October 2008 for the site D; 210 days) in order to obtain integrative fluxes concerning the vegetative period of *V. spiralis*. In doing so, daily rates were assumed to be constant during the three sampling macroperiods.

Photosynthetic quotients (PQ) were calculated as the absolute values of the ratio between O_2 and TCO_2 evolved and fixed in the light by *V. spiralis*; such fluxes from and to the plant fronds were obtained subtracting rates measured in bare sediments from those obtained in vegetated sediments (Pokorný et al., 1989). Respiratory quotients (RQ) were determined as the absolute values of the ratio between TCO_2 and O_2 fluxes measured in the dark in bare sediments (Rich, 1975; Granéli, 1979).

5.2.3 Porewater methane concentration

At the end of each incubation, the microcosm sediments were subsampled by means of a small core (i.d. 2 cm, height 10 cm) and interstitial water was extracted via centrifugation under nitrogen atmosphere (3000 rpm for 5 minutes). The supernatant was analyzed gas-chromatographically for dissolved methane with the headspace method (2 ml headspace into a 4 ml gas-tight vial).

5.2.4 *V. spiralis* net production

When incubations and porewater methane determination were finished, *V. spiralis* shoots were collected from each microcosm. Biomass was sorted into above and belowground shares, rinsed with *in situ* water and desiccated (70 °C until constant weight) for dry weight determination. Root:shoot ratio (RSR) was calculated as the proportion of belowground to aboveground biomass and expressed as ($\text{g}_{\text{dw}} \text{g}_{\text{dw}}^{-1}$).

In order to assign O₂ and TCO₂ fluxes to *V. spiralis* alone and compare the plant net production ($\mu\text{mol g}_{\text{dw}}^{-1}\text{h}^{-1}$) at the two sites, ecosystemic fluxes in vegetated microcosms were initially corrected for fluxes measured in bare sediments and then normalized for the macrophyte biomass (above+belowground). Net growth rates (NGR, $\text{g}_{\text{dw}} \text{g}_{\text{dw}}^{-1}\text{d}^{-1}$) were finally calculated from O₂ data, assuming an O₂ to CO₂ ratio of 1.2, as commonly reported in the literature (Kemp et al., 1986; Wetzel and Likens, 1991) and a conservative carbon content in *V. spiralis* of 0.31 g C $\text{g}_{\text{dw}}^{-1}$ (Pinardi et al., 2009). NGR from oxygen data were compared with rates calculated with TCO₂ data.

5.2.5 Statistical analyses

Differences between O₂, TCO₂ and CH₄ fluxes were statistically tested by means of ANOVA and Tukey's HSD multiple comparison test, with sites, seasons, light, and vegetation as factors. Analyses were performed using the R Program (R - Development Core Team, 2009); statistical significance was set at $p \leq 0.05$. Fluxes of O₂ and TCO₂ measured in the light and dark were tested separately in order to exclude any significance due to predictable photosynthetic performance by plants. All average values are reported with associated standard error (SE).

5.3 Results

5.3.1 General features of sediments and transplanted plants

Sediments at U and D sites were muddy and were mainly composed of recently deposited fine particles. At station D sediments had significantly lower density (ANOVA, $F=28.41$, $p < 0.0001$; Tukey's HSD, $p < 0.01$) and higher water content (ANOVA, $F=393.59$, $p < 0.0001$; Tukey's HSD, $p < 0.0001$) (Table 1). Moreover, the two sites significantly differed for organic matter content (ANOVA, $F=7.06$, $p < 0.01$). Upon recovery, visual check across the transparent microcosm walls revealed that unvegetated units presented dark sediments with the exception of an uppermost surface layer (<5 mm) that appeared light brown. Plants in

vegetated microcosms exhibited new leaves and looked extremely healthy, indicating that the manipulation had a negligible effect on *V. spiralis*. Additionally, at both sampling sites, all visible roots were surrounded by a 1-2 mm thick layer of brownish mud, suggesting the presence of oxidized niches within the reduced sediments. *V. spiralis* shoot density measured *in situ* was constant among seasons and similar at both sites (mean value 575 ± 87 shoots m^{-2} , pooled data, $n=18$). In vegetated microcosms, average biomass of transplanted plants was 413.5 ± 26.8 and 383.2 ± 38.2 $g_{dw} m^{-2}$ at U and D, respectively (pooled data from the three experiments, $n=24$) (Table 2); the biomass peak in the microcosms from site D probably reflected higher blade length at this site in summer. Calculated RSR was comprised between 0.17 ± 0.01 and 0.82 ± 0.06 and varied seasonally, with minimum values in summer (Table 2; ANOVA, $F=78.96$ $p < 0.0001$; Tukey's HSD, $p < 0.0001$); differences between sites were not significant.

Table 1. General features of the sediments employed in the three experiments ($n=4$, mean \pm SE).

Site	Season	Density ($g mL^{-1}$)	Porosity (%)	Water content (%)	OM content (%)
U	Spring	1.26 ± 0.01	0.75 ± 0.01	59.5 ± 0.1	6.6 ± 0.2
	Summer	1.36 ± 0.04	0.65 ± 0.02	47.7 ± 0.1	6.1 ± 0.1
	Autumn	1.37 ± 0.02	0.76 ± 0.01	55.3 ± 0.4	6.4 ± 0.1
D	Spring	1.50 ± 0.07	0.78 ± 0.04	52.2 ± 0.3	10.3 ± 0.3
	Summer	1.13 ± 0.01	0.80 ± 0.01	70.9 ± 0.1	10.5 ± 0.2
	Autumn	1.14 ± 0.01	0.82 ± 0.01	72.1 ± 0.5	9.9 ± 0.2

5.3.2 Dissolved oxygen and inorganic carbon fluxes

Rates of O_2 and TCO_2 exchange in the light and dark were about one order of magnitude higher in microcosms with *V. spiralis* compared to bare sediments, in particular during summer (O_2 , Light: ANOVA, $F=437.26$, $p < 0.0001$; Dark: ANOVA, $F=344.44$, $p < 0.0001$; TCO_2 , Light: ANOVA, $F=825.20$, $p < 0.0001$; Dark: ANOVA, $F=106.35$, $p < 0.0001$) (Figures 5.1 and 5.2). In the light, vegetated sediments of both sites evolved oxygen toward the water column and were net TCO_2 sinks, with rates comprised between 1.2 ± 0.5 and 26.7 ± 1.0 $mmol O_2 m^{-2} h^{-1}$ and between -3.6 ± 0.5 and -86.4 ± 2.5 $mmol TCO_2 m^{-2} h^{-1}$ (Net Ecosystem Production, NEP, Figures 5.1 and 5.2). In the light, inorganic carbon uptake in vegetated sediments was systematically higher than the corresponding oxygen production, with an O_2 to

TCO₂ flux ratio of 0.45±0.04 (pooled data). Bare sediments displayed in the light both positive and negative fluxes (from -1.3±0.1 to 1.5±0.6 mmol O₂ m⁻²h⁻¹ and from -2.0±0.9 to 1.8±0.7 mmol TCO₂ m⁻²h⁻¹); photosynthetic activity by microphytobenthos turned the sediment into a net O₂ source in spring, at U, and in spring and autumn, at D. TCO₂ fluxes measured in the light at U were opposite and stoichiometrically comparable to those of oxygen, while sediments at D were a TCO₂ sink in all experiments (Figure 5.2).

Table 2. Transplanted *V. spiralis* biomass (g_{dw} m⁻²) and root:shoot ratio (RSR) (g_{dw} g_{dw}⁻¹) (n=8, mean ± SE).

Site	Season	Total biomass (g _{dw} m ⁻²)	Aboveground (g _{dw} m ⁻²)	Belowground (g _{dw} m ⁻²)	RSR (g _{dw} g _{dw} ⁻¹)
U	Spring	497.3±23.2	303.4±20.5	193.9±10.1	0.66±0.06
	Summer	379.9±56.5	324.3±31.3	55.6±6.0	0.18±0.03
	Autumn	363.2±43.0	243.5±4.6	119.8±7.0	0.49±0.04
D	Spring	281.6±20.9	154.7±10.2	126.9±12.1	0.82±0.06
	Summer	589.2±54.0	503.4±27.6	85.8±6.9	0.17±0.01
	Autumn	278.9±43.9	197.5±33.4	81.4±11.2	0.44±0.05

Respiration rates in vegetated sediments (from -3.1±0.3 to -37.8±2.4 mmol O₂ m⁻²h⁻¹ and from 3.5±0.6 to 44.9±3.5 mmol TCO₂ m⁻²h⁻¹, Ecosystem Respiration, ER, Figures 5.1 and 5.2) displayed a seasonal pattern with a summer peak coinciding with higher water temperature. With the summer incubation of sediments at D as only exception, respiratory quotients (RQ) measured in the dark in bare sediments were stoichiometrically comparable, with a TCO₂:O₂ flux ratio of 1.13±0.09 (pooled data), suggesting a negligible accumulation of anaerobic metabolism end-products within porewater. This was not the case in the summer incubation of microcosms at D site, when calculated RQ was 1.8.

Overall, daily rates allowed to distinguish different patterns between bare and vegetated sediments and between O₂ and TCO₂ fluxes (Figures 5.1 and 5.2). Microcosms with bare sediments were mostly an oxygen sink, while microcosms with *V. spiralis* were a net oxygen source to the water column during summer, with rates of 33.8±34.4 and 60.7±26.5 mmol O₂ m⁻²d⁻¹ measured at U and D sites, respectively. In vegetated sediments, TCO₂ NEP and ER were nearly balanced in spring and autumn, while in summer NEP largely prevailed and the *V. spiralis* meadow turned into a significant inorganic carbon sink (-515.7±71.8 and -891.6±48.9 mmol TCO₂ m⁻²d⁻¹ at U and D, respectively). In vegetated sediments, the

stoichiometry of TCO₂ and O₂ daily budgets measured in summer was quite unbalanced with ratios comprised between 6 and 9 (absolute value). Unvegetated sediments were a net TCO₂ source to the water column in summer and autumn (from 10.4±12.8 to 48.4±11.9 mmol TCO₂ m⁻²d⁻¹), with daily rates stoichiometrically comparable to those of O₂ (Figures 5.1 and 5.2).

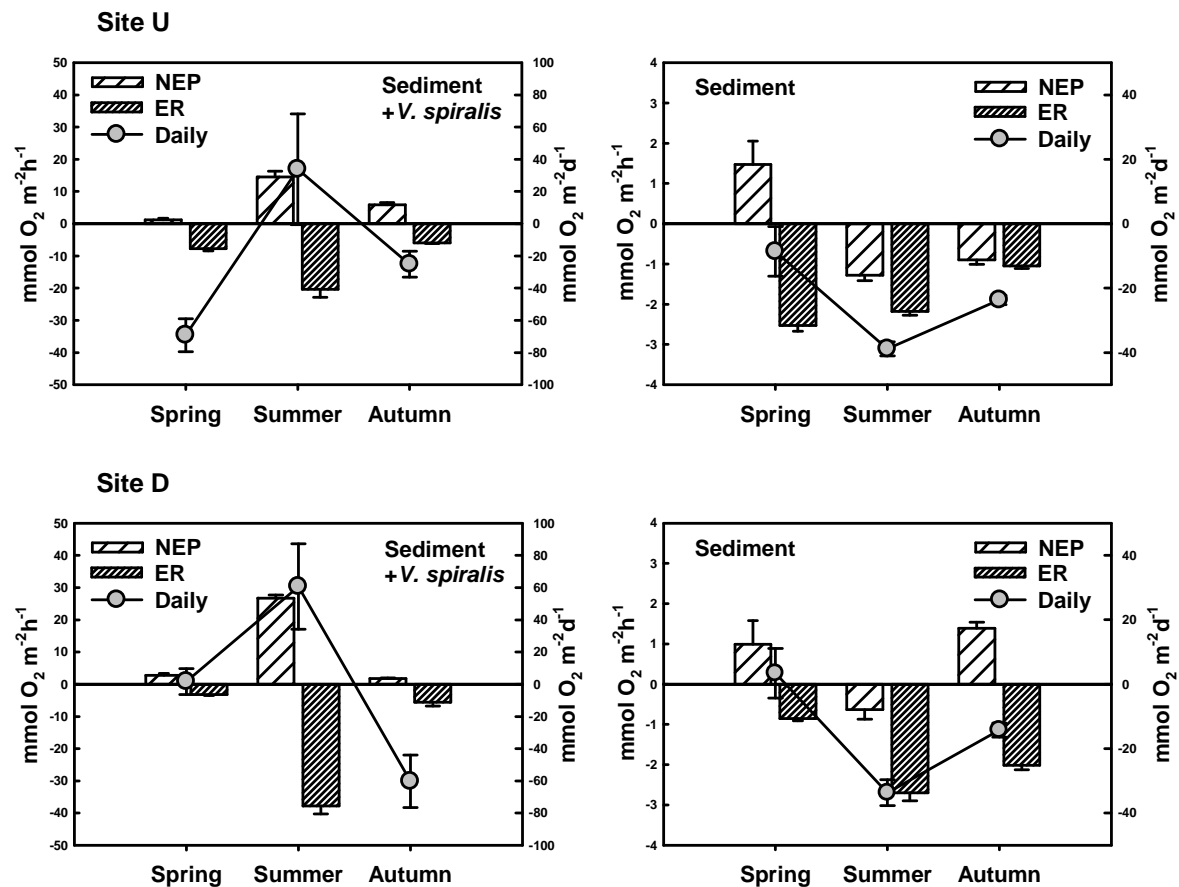


Figure 5.1. Hourly O₂ fluxes measured in the light (Net Ecosystem Production, NEP) and in the dark (Ecosystem Respiration, ER) and daily rates. Measurements were performed at two sites (U, graphs above and D, graphs below) and on *V. spiralis* vegetated (right) and bare sediment (left) microcosms (n=4, mean ± SE). Note the different scales between vegetated and bare sediment.

5.3.3 Methane fluxes and porewater concentrations

Methane fluxes were always directed to the water column with the only exception of rates measured during summer in vegetated sediments at D (Figure 7.3). Differences between methane fluxes mainly depended by the presence of *V. spiralis* (ANOVA, $F=17.42$, $p<0.0001$), but significant interaction term suggested the weight of the season and of the site (ANOVA, $F=4.51$, $p<0.05$); the factor light was instead not significant. At U, methane efflux

peaked in autumn in vegetated microcosms, while at D it peaked in summer in bare sediments, coinciding with maximum respiratory quotient (Figure 5.3); moreover, in summer, methane efflux from bare sediments at D was significantly higher than that measured at U (Tukey's HSD, $p < 0.01$).

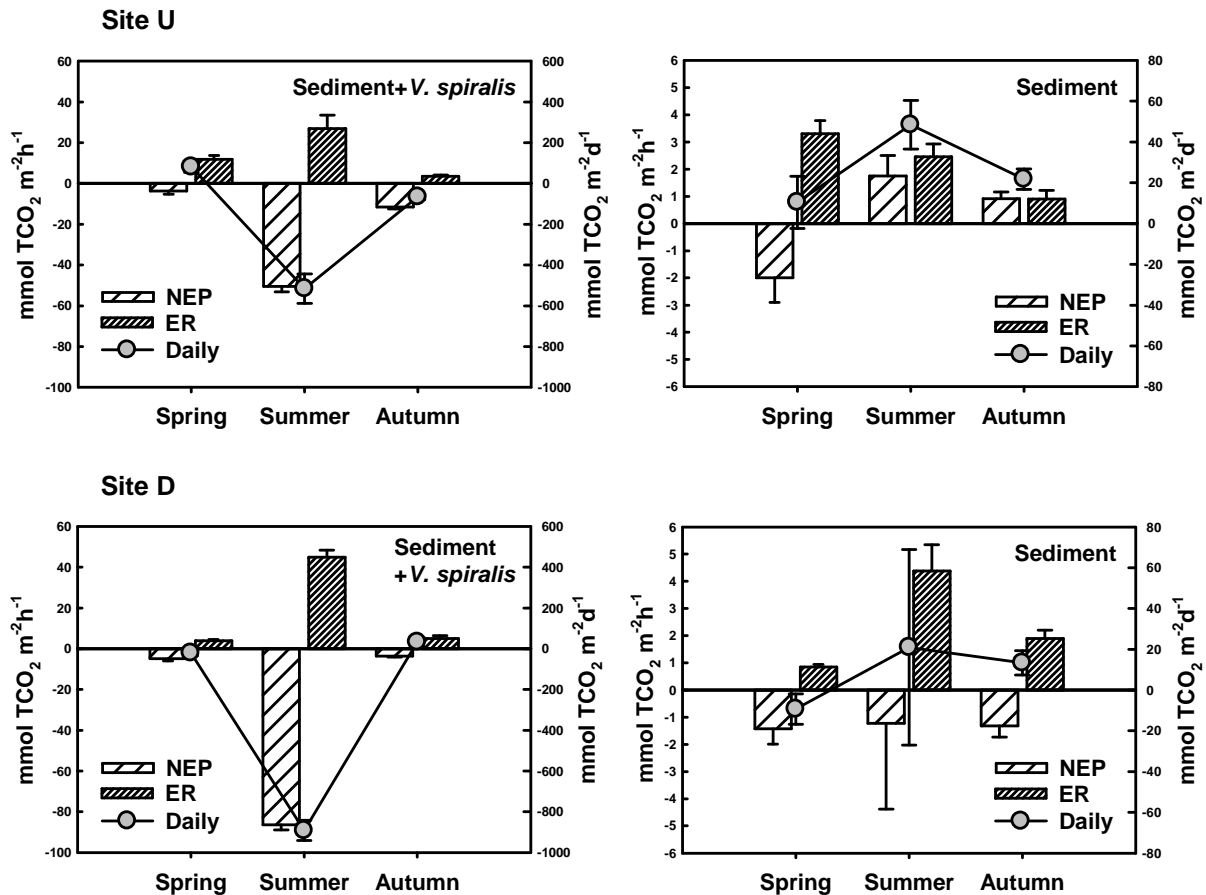


Figure 5.2. Hourly TCO₂ fluxes measured in the light (Net Ecosystem Production, NEP) and in the dark (Ecosystem Respiration, ER) and daily rates. Measurements were performed at two sites (U, graphs above and D, graphs below) and on *V. spiralis* vegetated (right) and bare sediment (left) microcosms ($n=4$, mean \pm SE). Note the different scales between vegetated and bare sediment.

On a daily basis, at both sites, unvegetated sediments were a net methane source (from 1.3 ± 0.3 to 6.8 ± 2.6 mmol CH₄ m⁻² d⁻¹) (Figure 3), whereas sediments with *V. spiralis* were both sources or sinks (from -2.4 ± 0.3 to 3.0 ± 0.6 mmol CH₄ m⁻² d⁻¹) (Figure 5.3). Methane concentrations measured in sediment porewater exhibited a strong seasonality with summer peaks (ANOVA, $F=38.79$, $p < 0.001$; Tukey's HSD, $p < 0.01$). In vegetated sediments, dissolved CH₄ concentrations were comprised between 0.6 ± 0.5 and 17.3 ± 16.1 μ M and they were generally lower than those measured in bare sediments (between 31.2 ± 5.9 and 96.2 ± 8.2

μM and between 29.8 ± 2.7 and 164.6 ± 9.9 μM , at U and D, respectively) (Figure 5.4; ANOVA, $F=329.81$, $p < 0.001$). Significant interaction term suggested that differences between vegetated and bare sediments depended upon sampling sites and sampling seasons but were not influenced by the light/dark condition (ANOVA, $F=22.21$, $p < 0.001$).

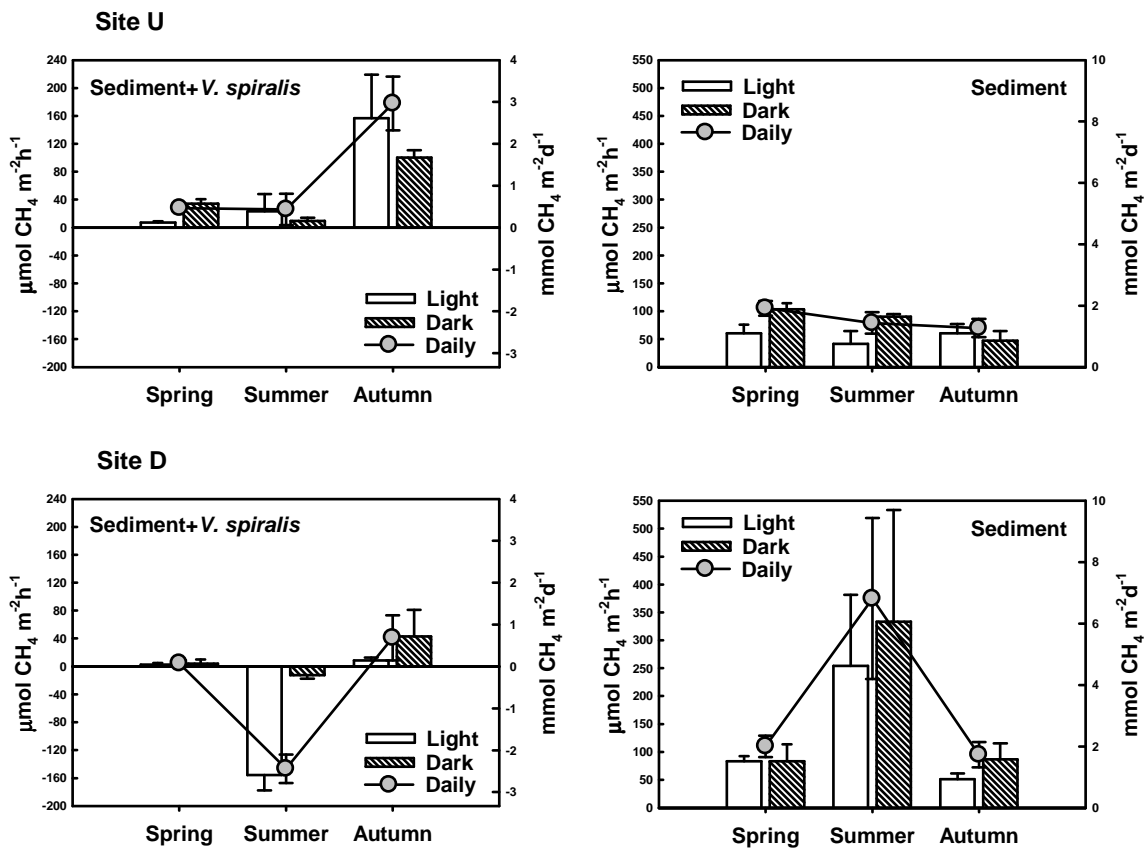


Figure 5.3. Hourly methane fluxes measured in the light and in the dark conditions, and daily rates. Measurements were performed at two sites (U, graphs above and D, graphs below) and on *V. spiralis* vegetated (right) and bare sediment (left) microcosms ($n=4$, mean \pm SE).

5.3.4 *V. spiralis* net production and respiration

Ecosystemic O_2 and TCO_2 fluxes corrected for the contribution of sediments and normalized for the *V. spiralis* biomass are reported in Table 3, together with photosynthetic quotients.

The highest net production and respiration rates (absolute values) were calculated for the summer sampling period, when irradiance and water temperature stimulated the plant metabolic activities; differences between sites were not significant. The respiration of *V. spiralis* estimated with O_2 and TCO_2 fluxes was comparable, as rates calculated with the two methods (absolute values) were not significantly different. In the light, TCO_2 fluxes (absolute values) were 1.5 to 3 folds higher than the corresponding O_2 fluxes; calculated PQs were

consistent between sampling sites and varied seasonally between 0.20 and 0.86 with a summer minimum (Table 3; ANOVA, $F=14.05$, $p<0.001$; Tukey's HSD, $p<0.01$).

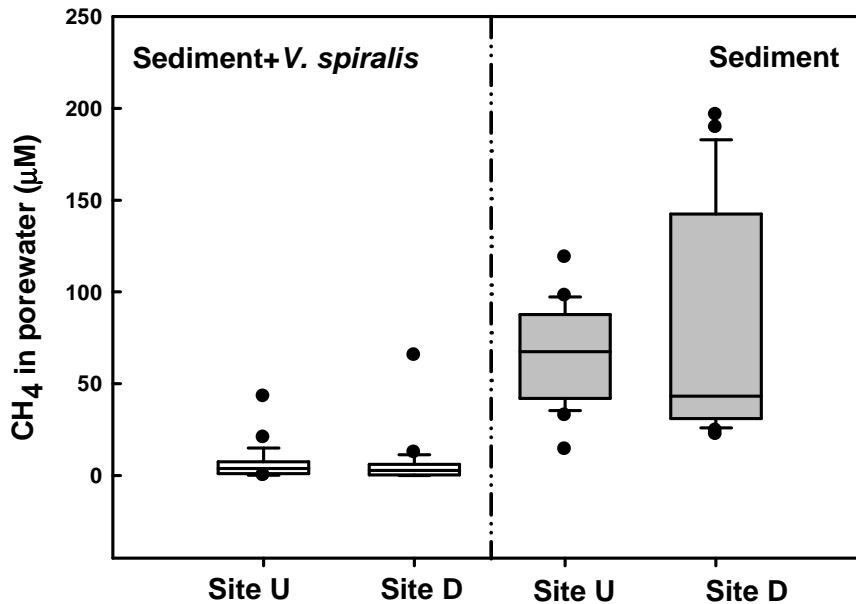


Figure 5.4. Methane concentrations in the porewater of *V. spiralis* vegetated (left) and bare sediments (right) collected at sites U and D. Each boxplot comprises data from three experimental periods (n=24).

Net *V. spiralis* growth rates, obtained from O₂ and TCO₂ normalized fluxes, ranged from 0.004 ± 0.001 to 0.032 ± 0.005 g_{dw} g_{dw}⁻¹d⁻¹ (O₂ data) and from 0.006 ± 0.001 to 0.088 ± 0.016 g_{dw} g_{dw}⁻¹d⁻¹ (TCO₂ data). Growth rates were not statistically different at the two sampling sites. Calculations based on O₂ and TCO₂ data resulted in similar net growth estimates in spring and autumn but not in summer, when net growth rate calculated with TCO₂ values was approximately two folds higher than that calculated with O₂ fluxes (Figure 5.5; Tukey's HSD, $p<0.01$).

Table 3. Light and dark O₂ and TCO₂ exchange normalized for *V. spiralis* total biomass and Photosynthetic Quotients (n=4, mean ± SE).

Site	Season	Normalized fluxes (μmol g _{dw} ⁻¹ h ⁻¹)				Photosynthetic Quotient O ₂ :TCO ₂
		O ₂ LIGHT	TCO ₂ LIGHT	O ₂ DARK	TCO ₂ DARK	
U	Spring	7.3±1.2	-13.5±3.0	-10.9±1.1	17.6±3.0	0.58±0.09
	Summer	48.4±7.0	-160.5±29.2	-71.4±37.1	99.8±58.4	0.32±0.05
	Autumn	20.5±4.6	-35.0±4.0	-16.1±4.0	7.8±2.3	0.58±0.11
D	Spring	13.5±1.1	-21.2±1.7	-7.8±0.8	10.5±1.1	0.64±0.03
	Summer	47.3±4.3	-148.6±21.6	-69.9±11.7	85.2±22.0	0.33±0.02
	Autumn	21.8±2.1	-31.9±4.2	-9.3±2.6	7.6±3.5	0.69±0.04

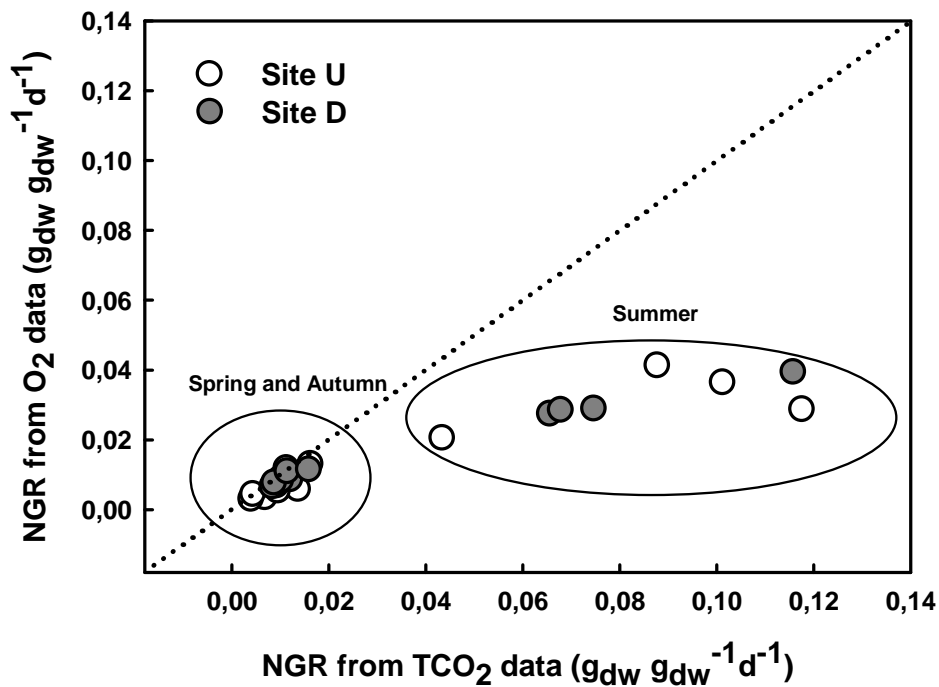


Figure 5.5. Comparison between *V. spiralis* net growth rates (NGR) calculated from O₂ and TCO₂ data.

5.4 Discussion of the results

5.4.1 Benthic ecosystem metabolism

In the Mincio River, *V. spiralis* is growing on muddy substrates arising from local flow reduction and particulate sedimentation, processes that are enhanced by macrophytes with a positive feedback (Madsen et al., 2001; Li and Xie, 2009). The accumulation of fine organic particles on the river bottom likely increases the sedimentary demand of electron acceptors and promotes reducing conditions, especially during summer, when water temperature is elevated. In this context, oxygen transport is necessary for root respiration and to detoxify porewater, buffering the buildup of anaerobic metabolism end-products that can menace the plants survival. This physiological adaptation, together with elevated rates of primary production, have relevant direct and indirect effects on the benthic metabolism. Assuming constant rates in spring, summer and autumn, we extended O_2 , TCO_2 and CH_4 fluxes to the whole experimental period (210 days, Figure 5.6). At both sites *V. spiralis* played a major role in driving inorganic carbon, oxygen and methane benthic exchanges. Vegetated sediments were an efficient trap for TCO_2 , whereas bare sediments resulted net heterotrophic. At U, both vegetated and unvegetated sediments were net oxygen sinks; however, oxygen consumption was halved within the meadow, where the occurrence of the plants partially counterbalanced the sedimentary oxygen demand. At D, vegetated sediments were a net source of oxygen, mostly due to summer production, in contrast with the net consumption in bare sediments.

Concentrations of porewater CH_4 were significantly attenuated in the presence of *V. spiralis* at both sites; coherently, methane efflux from vegetated sediments was significantly reduced or reversed, likely due to inhibition of methanogenesis or to enhanced methanotrophy (Figure 5.6). This is in agreement with CH_4 flux measurements effectuated on a meadow of *V. gigantea*, which resulted constantly lower than those measured on unvegetated areas (Boon and Sorrell, 1991). The lack of significantly different CH_4 fluxes in the light and dark conditions allows us to exclude any relevant lacunar CH_4 transport from leaves, in contrast with results reported for *Myriophyllum exalbescens* by Heilman and Carlton (2001). This outcome could be attributable to the accumulation of oxygen within the sediment during daylight, and thus elevation of rhizospheric Eh, sufficient to control methanogenesis also during night time (Jaynes and Carpenter, 1986; Boon and Sorrell, 1991; Wigand et al., 1997).

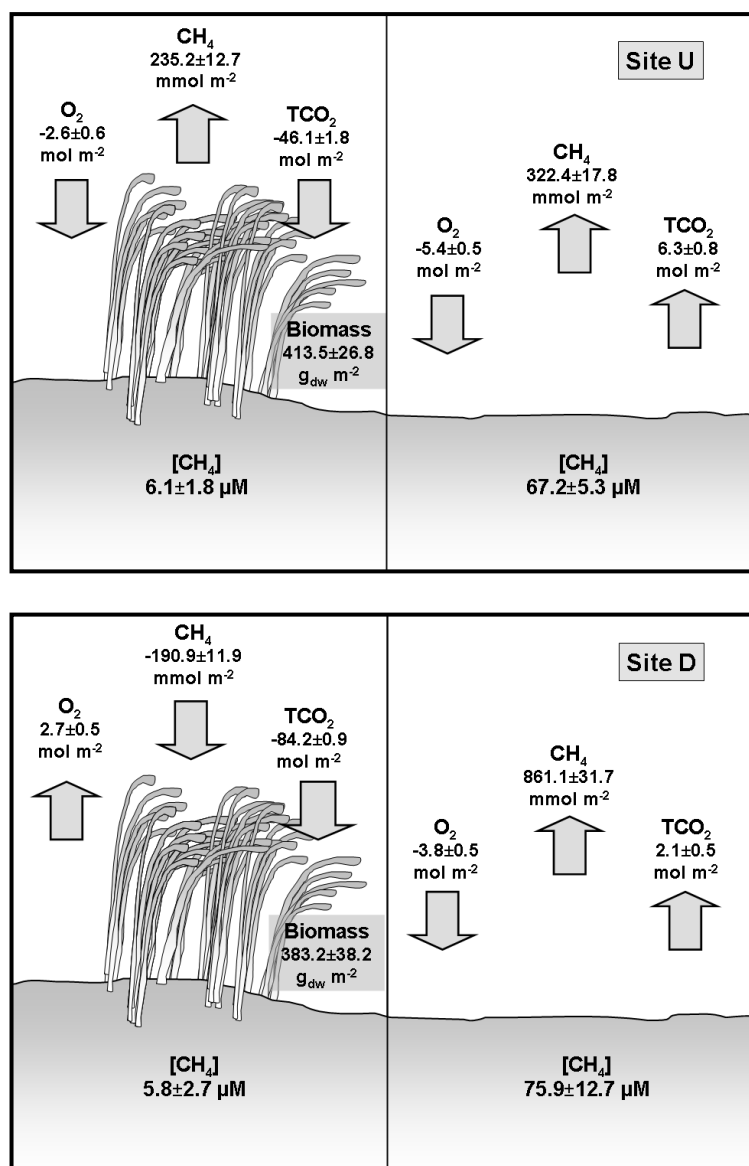


Figure 5.6. O_2 , TCO_2 and CH_4 exchanges across the *V. spiralis* vegetated and bare sediment interface calculated during the vegetative period (April–November 2008) at sites U (above) and D (below). *V. spiralis* mean biomass and porewater CH_4 mean concentrations are also reported.

Methanotrophs communities colonizing the roots and stolons of the plants are likely playing an important role in the limitation of methane release from sediment to the water column (Sorrell et al., 2002), as well as epiphytic communities associated to elevated blade density (Heilman and Carlton, 2001). Wigand et al. (1997, 2000) also reported how *V. americana* significantly influences the benthic-pelagic system by direct (e.g. nutrient uptake) and indirect effects (e.g. modification of rhizospheric Eh). Dissimilative microbial processes with higher energy yield are likely occurring in the anoxic sediments adjacent to the oxic

microniches around roots. In this case, radial oxygen loss promotes some deep aerobic respiration, far from the uppermost oxic sediment layer, and the oxidation of anaerobic metabolism end-products. This could indicate that redox processes are more coupled in vegetated sites compared to bare sediments where processes are vertically confined. There are diverse bacterial processes that could interfere with methanogenesis in presence of oxygen. When retrieving microcosms from the river bottom, we had already observed oxygen leakage from the roots resulting in visible formation of the solid phase Fe(III) oxyhydroxides. Iron reduction could be one of those competing with methanogens for organic carbon substrate, in a coupled iron oxidation-reduction system (Roden and Wetzel, 1996; Neubauer et al., 2005). Oxygen leakage to sediments can moreover enhance coupled nitrification-denitrification (Risgaard-Petersen and Jensen, 1997, Ottosen et al, 1999; Pinardi et al., 2009).

*5.4.2 Linking photosynthetic quotient and ROL in *V. spiralis**

A few studies report experimental measurements of photosynthetic quotients in submersed macrophyte communities (Kemp et al., 1986; Pokorný et al., 1989) and most authors rely on a ~1:1 stoichiometry between O₂ production and TCO₂ uptake. Results reported in the present study demonstrate that PQ calculated from O₂ and TCO₂ fluxes measured via incubation of intact plants rooted in sediments is far below the unit and varies considerably within the vegetative period, as *in situ* conditions strongly affect the amount of oxygen transferred to the rhizosphere and thus the fraction evolved to the water column.

PQs calculated from vegetated microcosms incubation surely underestimate the real balance between O₂ production and TCO₂ fixation, and the degree of underrating is directly proportional to the amount of oxygen transferred belowground by *V. spiralis*. As a consequence, the distance of PQ measured on plants rooted in sediments from the ~1 theoretical value is probably positively correlated with the plant ROL. Our results suggest that *V. spiralis* varies seasonally the quota of oxygen transferred to the roots, with relatively minor amounts in spring and autumn and much transport in summer, coinciding with the highest sediment respiration rates and minimum PQ. Of course this kind of approach allows to produce only qualitative results: the actual amount of oxygen transferred to the rhizosphere should be directly measured on the porewater.

Results from this study suggest that, when incubating intact plants rooted in sediments, oxygen-based methods can bring to significant underestimation of net primary production, making direct comparison inappropriate with other experimental approaches. Photosynthetic rates measured on macrophytes leaves for example are one order of

magnitude higher than those reported in the present study on intact plants (Nielsen and Sand-Jensen, 1989). Primary production and growth rate estimates via TCO₂ based methods seem on the contrary more reliable. Growth rates obtained in the present study from inorganic carbon fluxes show indeed a good agreement with those obtained with direct measurements on *Vallisneria* spp. in other studies (Table 4).

5.4.3 *V. spiralis* adaptations to OM content and sedimentary respiration

In this study we hypothesized that the fraction of photosynthetically produced oxygen transferred to the rhizosphere would be proportional to the organic matter content of sediments and variable within the vegetative period. We did not measure ROL directly and we suggested to use the imbalance between O₂ and TCO₂ flux data (the distance of calculated PQs from the ~1 theoretical value) as a proxy of radial oxygen loss. Our results indicate that at the two investigated sites, differences in sediment organic matter (6 and 10%, at U and D respectively) were not reflected by significant differences between calculated PQs. On the contrary, significantly lower PQs values were calculated at both sites in the summer period; in the considered range of OM, ROL was thus not affected. Influences of sedimentary fertility (nutrients and organic matter availability) on *Vallisneria* spp. have been reported by Wang and Yu (2007), Xiao et al. (2007) and Xie et al. (2007): results indicated that this genus is stimulated in terms of roots and ramets production in nutrient-rich patches (up to 13% as LOI). Those outcomes indicate a net contrast with the physiology of submersed macrophytes colonizing oligotrophic systems, that are generally depressed by increasing OM (Barko and Smart, 1986; Sand-Jensen et al., 2005).

Root:shoot ratio values measured on *Vallisneria* spp. in various studies are reported in Table 4; our results fall within the upper part of the overall range. As we did not observe any change in OM content along the year, we cannot attribute RSR temporal variations to nutrient limitation. We rather think that the reduction of the root biomass during summer is connectable to the increasing respiration rates within the sediment, and thus to an elevate sedimentary oxygen demand. The plant should therefore reduce the surface of tissue that could loose oxygen through diffusion. Moreover, as lower RSRs correspond to lower PQs, a reduced quantity of roots releases an higher fraction of oxygen to the rhizosphere. This is likely the combination of two plastic physiological adaptations that allow *V. spiralis* to be at ease in colonizing eutrophic sediments.

Table 4. Reported growth rates and root-to-shoot ratios (RSR) for *Vallisneria* spp.

Site	Species	System	Study	Period	Growth rate ($g_{dw} g_{dw}^{-1} d^{-1}$)	Method	Root:shoot ratio	Authors
Chesapeake Bay, Maryland (USA)	<i>V. americana</i>	Intertidal Lagoon	Harvesting	Summer 1990, 1993, 1995	-	-	0.16-0.18	Wigand et al., 1997
Davie, Florida (USA)	<i>V. americana</i>	Laboratory	Cultivation at low/high soil fertility	Summer 1994	-	-	0.23-0.47	Van et al., 1999
Blanchetown, South Australia	<i>V. americana</i>	Laboratory	Cultivation at low/high irradiance	Winter 1995	0.02-0.10	Leaf-Marking	0.12-0.27	Blanch et al., 1998
Hubei Province, China	<i>V. natans</i>	Laboratory	Cultivation in different nutrient-rich sediment and water	Spring 2004	0.02-0.09	Biomass variation measurements	0.08-0.22	Xie et al., 2005; 2007
Hubei Province, China	<i>V. natans</i>	Laboratory	Cultivation in different sediment type	Spring 2004	-	-	0.05-0.55	Xiao et al., 2007
Hubei Province, China	<i>V. spiralis</i>	Laboratory	Cultivation at low/high soil fertility	April-July 2004	-	-	0.18-0.40	Wang & Yu, 2007
Citrus County, Florida	<i>V. americana</i>	Intertidal Lagoon	<i>In situ</i> measurements	May 2001-May 2002	0.01-0.02	Leaf-Marking	0.08-0.50	Hauxwell et al., 2007
Lombardia, Italy	<i>V. spiralis</i>	River	<i>In situ</i> measurements	April-December 2007	0.01-0.08	Leaf-Marking	0.27-0.51	Pinardi et al., 2009
Hubei Province, China	<i>V. spiralis</i>	Laboratory	Cultivation in different sediment type	-	0.05-0.09	Biomass variation measurements	0.06-0.11	Li & Xie, 2009
Lombardia, Italy	<i>V. spiralis</i>	Laboratory	Incubation for flux measurements	April-November 2008	0.01-0.09	Gas exchange (TCO_2)	0.17-0.82	This study

6. Effects of the early colonization of a submerged macrophyte (*Vallisneria spiralis*) on porewater sediment chemistry

6.1 Aim of the study

Roots of submerged macrophytes can deeply affect the microenvironment within the surface and deep sediments they colonize, as a consequence of direct, i.e. solute uptake, and indirect effects, i.e. radial oxygen loss (Carpenter et al., 1983; Karjalainen et al., 2001). Submerged macrophytes rely primarily on sediments for assimilation, since the available nutrient concentration is generally much higher in pore water than in the water column (Barko et al., 1991; Carr & Chambers, 1998). Assimilation by plants has the potential to deplete sedimentary ammonium and reactive phosphorus pools, attenuating their recycling to the water column and their availability to other primary producers (Wigand et al., 2000). Furthermore, the presence of roots generally enhances the iron bound, solid-phase pool of PO_4^{3-} , augmenting the P retention capacity of the sediment (Wigand et al., 1997; Hupfer & Dollan, 2003).

The aim of the present study was to analyse the early stage of sediment colonization by the roots of a submerged macrophyte (*Vallisneria spiralis* L.) and to investigate its short-term effects at two sites differing for the content of organic matter in sediments and nitrate availability in the water column. *V. spiralis* presents ribbon-shaped leaves, expands through stolons forming monospecific beds, colonizes both stagnant and lotic freshwater environments and a variety of substrates, from gravel to muddy, organic rich bottom (Wang & Yu, 2007; Li & Xie, 2009; Pinaridi et al., 2009).

We hypothesized that the macrophytes control pore water chemistry through the ROL and that ROL effects are in turn influenced by organic matter enrichment of sediment. We expected minor oxygen release at increasing organic content, resulting in limited capacity of the plant to detoxify pore water from anaerobic metabolism end products. We also hypothesized that nitrate availability in the water enhances nitrogen uptake from the leaves and attenuates nitrogen uptake from the sediment, resulting in accumulation of inorganic nitrogen in the rhizosphere.

6.2 Sampling program and calculations

6.2.1 Experimental setup

On the 9th of March 2009, sediment, water and specimens of *V. spiralis* were collected from two different sites of the Mincio River (Northern Italy), upstream (site 22) and downstream (site 23) the urban wastewater treatment plant of the Lake Garda district, that processes 330.000 inhabitant equivalents. At both sampling sites sediments were muddy and presented patchy meadows of *V. spiralis*. Approximately 5 l of unvegetated sediment, 200 l of water and about 100 shoots of *V. spiralis* were collected at each site. Sediments were collected with plexiglas liners and only the upper 0-5 cm horizon was sliced and used; *V. spiralis* shoots were gently collected digging by hand the sediment around the rhizosphere, in order to minimize root damage. The period of the experiment coincided with the beginning of the vegetative phase of *V. spiralis*, when the submerged mats expands and new stolons colonize bare sediments (Pinardi et al., 2009). In the Mincio River, water flow and nutrient availability are generally elevated in March, due to precipitation, runoff and no water withdrawal for irrigation.

In laboratory, within 2 hours from collection, the sediment from each site was sieved (0.2 cm mesh) to remove large animals and plant fragments, and homogenised; the obtained slurries were transferred into cylindrical plexiglas tubes (i.d. 3 cm, height 10 cm). Three sediment subsamples were collected from each slurry for characterization. Shoots of similar size were carefully washed with river water to remove epiphytes from leaves and sediment particles from roots; one shoot was then transplanted into each tube. Overall, 96 microcosms were set up, of which 24 tubes with plants and 24 with bare sediments from upstream (U_V , upstream vegetated sediment; U_B , upstream bare sediment); 24 with plants and 24 with bare sediment from downstream (D_V , upstream vegetated sediment; D_B , upstream bare sediment). Microcosms were then incubated in two tanks with river water from U and D, and maintained at 19°C with a 12/12 light/dark cycle at 300 $\mu E m^{-2}s^{-1}$ irradiance. Water was stirred and oxygenated with aquarium pumps (Figure 8.1).

From the 10th of March to the 4th of April 2009 (25 days), approximately every 4 days, 3 replicates for the each experimental condition (U_B , D_B , U_V and D_V) were terminated, in order to monitor changes in pore water chemistry upon initial conditions. In the microcosms with macrophytes, leaves were cut at the base, the upper sediment layer (0-5 cm, containing most roots biomass) was extruded and squeezed under N_2 atmosphere and the obtained pore water was immediately analysed for Eh, pH, O_2 , DIC, CH_4 , Fe^{2+} , PO_4^{3-} , NH_4^+ , NO_3^- and DRSi. Leaves and roots were recovered for further analyses. Simultaneously to pore water analyses, water samples from each tank were analysed (temperature, Eh, pH, O_2 , CO_2 , PO_4^{3-} , NH_4^+ ,

NO₃⁻ and DRSi) and 20% of the water volume was replaced with fresh river water from the corresponding site.



Figure 6.1. Transplant of *V. spiralis* shoots and incubation tank in laboratory.

6.2.2 Porewater analyses

Dissolved O₂ concentration was measured with polarographic microsensors (Unisense, DK) connected to a picoamperometer (PA2000, Unisense, DK). Redox potential and pH were measured with potentiometric electrodes (Radiometer, DK) connected to a high impedance mV-meter (Crison micro pH 2002, ES). Dissolved inorganic carbon (DIC), dissolved carbon dioxide (CO₂) and dissolved methane (CH₄) were analysed according to methods described in chapter 4, by transferring 1 ml of sample into a 12 ml glass vials. All nutrients (PO₄³⁻, NH₄⁺, NO₃⁻ and DRSi) were analysed with standard spectrophotometric techniques described in A.P.H.A. (1981).

6.2.3 Sediment and plant analyses

Slurries from the two sites were immediately analysed for bulk sediment density, porosity and organic matter content, according to methods reported in chapter 4. Total N was determined with an elemental analyser (EA 1110, Carlo Erba) and total P following Aspila et al. (1976). Sediment samples (typically 5-10 mg) were used for measurements of ¹⁵N (see later).

Leaves and roots recovered from terminated microcosms were washed separately with river water, pooled and dried at 50°C until constant weight. Samples of dry leaves and roots were ground and sieved through a 0.2 mm mesh to ensure homogeneity of the sample. For each sample, approximately 0.05 mg and 2 mg were processed, respectively, for total C and total

N, including ^{15}N , with an elemental analyser (EA 1110, Carlo Erba) online with a mass spectrometer (Delta Plus XP, ThermoFinnigan).

Nitrogen isotope abundance is reported as $\delta^{15}\text{N}$ and calculated as follows:

$$\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where R is the $^{15}\text{N}/^{14}\text{N}$ ratio in the sample and in the atmospheric N_2 . The standard deviation of replicates was in general lower than $\pm 0.2\text{‰}$.

6.2.4 Statistical analyses

Differences between plants and sediments collected from the sites U and D were tested with one way ANOVA. The evolution of pore water variables in vegetated and unvegetated sediments of both sites was compared by means of ANCOVA (ANalysis of COVariance). For this purpose, the covariance of variables (pH, CO_2 , O_2 , Eh, CH_4 , NH_4^+ , SiO_2) versus time was tested for the four experimental conditions (U_B , D_B , U_V and D_V) with R statistical package (R Development Core Team, 2005). A priori contrasts were used to compare slopes and intercepts of the linear models.

6.3 Results

6.3.1 Water, sediment and plant features

As during the experiment part of the water in the two incubation tanks was regularly replaced with fresh river water from the experimental sites, the physico-chemical parameters remained rather constant for the whole 25 days period (Table 1). NH_4^+ , PO_4^{3-} , SiO_2 , DIC, O_2 , Eh and pH were not statistically different in the two tanks; NO_3^- , the dominant form of dissolved inorganic nitrogen (DIN), was 4-fold higher in D than U, likely due to the release of nitrate-rich waters from the wastewater treatment plant. NH_4^+ was with 10 and 4% at U and D a minor fraction of DIN. Despite water stirring, CO_2 was constantly supersaturated at both sites with higher values at D. Water in the two tanks was well oxygenated, with positive redox potentials and undetectable dissolved CH_4 (data not shown).

Table 1. Characteristics of the water in the two incubation tanks; (mean±SE, n=8) of the whole incubation period (25 days, from 03/10/09 to 04/04/09) are reported.

	Temperature (°C)	pH	CO ₂ (%)	O ₂ (%)	Eh (mV)	NO ₃ ⁻ (μM)	NH ₄ ⁺ (μM)	PO ₄ ³⁻ (μM)	SiO ₂ (μM)
U	19.6±0.6	8.3±0.1	506±47	95.5±1.7	260.4±20.8	18.4±1.9	2.4±1.3	0.3±0.3	15.3±0.7
D	19.4±0.6	8.1±0.1	872±69	90.6±0.8	249.4±9.9	77.8±2.6	2.9±1.6	0.4±0.2	23.6±0.5

At both sampling sites sediments were muddy and had similar density. Downstream the sewage plant sediments were more organic and were characterised by higher N and P content and had higher $\delta^{15}\text{N}$ abundance compared to upstream sediments (Table 2).

V. spiralis grew and new leaves replaced those senescent; shoot biomass did not change significantly during the course of the experiment and was similar in the two experimental conditions (Table 3). Root to shoot mass ratios were also similar and averaged 0.42 ± 0.13 . Total C and N content in *V. spiralis* was not statistically different between sites (ANOVA, $P>0.05$); N content tended to be lower in roots than in leaves. The ratio of total C and N in macrophyte tissue did not change significantly during the course of the experiment meaning that DIN in water and pore water was sufficient to ensure plant growth without N dilution in the plant biomass. Significantly higher $\delta^{15}\text{N}$ values were found in both leaves and roots at site D, indicating the N enrichment from urban wastewater (ANOVA, $P<0.05$, Table 3). Accordingly, total P content was significantly higher in leaves and roots of plants collected at site D than at site U (ANOVA, $P<0.05$, Table 3).

Table 2. Density, porosity, organic matter (OM), total N, fraction of the ¹⁵N isotope within total N and total P in the slurries realized with sediment collected from the 2 sampling sites on 03/09/09 and used for microcosms set up (mean±SE, n=3).

	Density (g cm ⁻³)	Porosity (%)	OM (%)	N (%)	$\delta^{15}\text{N}$ (‰)	P (%)
U	1.2±0.0	0.4±0.0	6.2±0.3	0.23±0.01	3.91±0.60	0.05±0.00
D	1.3±0.0	0.4±0.0	11.0±0.1	0.58±0.01	7.14±0.02	0.07±0.00

Table 3. Shoot biomass, total C, N and P, $\delta^{15}\text{N}$ and C:N:P molar ratio in leaves and roots of *V. spiralis* collected from U and D microcosms (mean \pm SE, n=7). U_L= upstream site, leaves; U_R= upstream site, roots; D_L= downstream site, leaves, D_R= downstream site, roots.

	Shoot biomass (g _{dw} shoot ⁻¹)	C (g l*100) g _{dw} ⁻¹	N (g l*100) g _{dw} ⁻¹	$\delta^{15}\text{N}$ (‰)	P (g l*100) g _{dw} ⁻¹	C:N:P (mol:mol)
U	0.15 \pm 0.02	31.6 \pm 1.7	3.0 \pm 0.1	4.4 \pm 0.3	0.31 \pm 0.0	264:21:1
L						
U	0.06 \pm 0.01	31.6 \pm 0.9	2.5 \pm 0.2	3.9 \pm 0.5	0.29 \pm 0.0	281:19:1
R						
D	0.16 \pm 0.02	33.8 \pm 0.9	3.1 \pm 0.1	9.9 \pm 0.4	0.48 \pm 0.1	182:14:1
L						
D	0.06 \pm 0.01	33.3 \pm 1.5	2.6 \pm 0.1	11.1 \pm 0.7	0.50 \pm 0.1	172:12:1
R						

6.3.2 Pore water chemistry in bare and vegetated sediments

Temporal changes of the pore water constituents are reported in figures 6.1 and 6.2; all pore water data integrate the uppermost 5 cm sediment layer, that includes the generally oxic sediment-water interface and most of the rhizosphere. The analyses of the covariance of pH, CO₂, O₂, Eh, CH₄, NH₄⁺ and DRSi versus time in the four experimental conditions U_B, D_B, U_V and D_V are summarized in the tables 4 and 5. Here we report the statistics of the linear regressions between pore water constituent and time (Table 4) and statistics of the comparison between slopes (contrast analysis) in the different experimental conditions (Table 5). We excluded from the analyses NO₃⁻ and PO₄³⁻ as their concentration did not change linearly in the experimental period. During the course of the incubation, pH decreased slightly in all treatments, with lower values in vegetated sediments and downstream. Accordingly, dissolved CO₂ concentrations were higher at site D and in vegetated sites compared to bare sediments.

Both sedimentary organic matter content and colonization by *V. spiralis* significantly affected O₂ evolution in the pore water, as evidenced by clear differences among treatments and sampling sites, with U_V>U_B>D_V>D_B. In U_V microcosms, average pore water O₂ concentration varied in the range of 20-40 μM , whilst in D_V O₂ increased almost linearly from 0 up to 20 μM . By contrast, in D_B O₂ was persistently below 5 μM . Eh values underwent similar trends, with a clear distinction between U and D, and vegetated and bare sediments. Overall, in D_B microcosms, the accumulation in pore water of anaerobic

metabolism end-products resulted in $Eh < -50$ mV, whilst in U_V persistent oxidizing conditions were evidenced by $Eh > +50$ mV.

Table 4. Linear regression of pore water variables versus time in the four experimental conditions (U_B =upstream bare sediment; U_V =upstream vegetated sediment by *V. spiralis*; D_B = downstream bare sediment, D_V =downstream vegetated sediment by *V. spiralis*, NS=not significant)

	Condition	Intercept	Slope	P	R ²
pH	U_B	7.27	-0.01	<0.01	0.35
	U_V	7.20	-0.01	<0.001	0.62
	D_B	7.07	-0.01	<0.001	0.85
	D_V	7.03	-0.01	<0.001	0.82
CO ₂	U_B	0.54	0.02	<0.01	0.24
	U_V	0.65	0.02	<0.01	0.35
	D_B	0.72	0.05	<0.001	0.64
	D_V	0.89	0.04	<0.001	0.58
O ₂	U_B	7.38	0.50	<0.01	0.27
	U_V	13.87	0.78	<0.05	0.23
	D_B	-	-	NS	-
	D_V	0	0.73	<0.001	0.69
Eh	U_B	130	-5.69	<0.001	0.65
	U_V	127	-2.14	<0.05	0.14
	D_B	-	-	NS	-
	D_V	-39	3.43	<0.001	0.51
CH ₄	U_B	-	-	NS	-
	U_V	329	-14.29	<0.001	0.73
	D_B	-	-	NS	-
	D_V	338	-14.08	<0.001	0.72
NH ₄ ⁺	U_B	-	-	NS	-
	U_V	310	-12.96	<0.001	0.81
	D_B	270	9.79	<0.001	0.50
	D_V	300	-9.61	<0.001	0.70
DRSi	U_B	0.20	0.003	<0.001	0.44
	U_V	-	-	NS	-

D _B	0.45	0.005	<0.05	0.22
D _V	-	-	NS	-

At both U and D, dissolved CH₄ concentrations exhibited a distinct pattern in bare and vegetated sediments, with rather constant and elevated values in the pore water of sediments without plants, up to 700 μM, and progressive disappearance of this gas in sediments colonised by *V. spiralis* (Fig. 6.2, Tables 4 and 5). Macrophytes exerted a significant control also on NH₄⁺ concentrations, that decreased progressively to almost undetectable values in U_V and D_V, whereas they were rather constant in U_B and D_B.

Dissolved Fe²⁺ and PO₄³⁻ concentrations were undetectable or extremely low in most analysed samples. The patterns of Fe²⁺ were rather erratic for D_V, U_V and U_B, with occasional accumulation during the course of the experiment. On the contrary, the concentration of the Fe²⁺ increased at D_B progressively from day 14 to the end of the experiment. PO₄³⁻ concentrations were generally below 5 μM and tended to decrease to about 1 μM in the pore water of D_V, U_V and U_B; in D_B concentrations were higher and exhibited an increasing pattern in the last part of the experiment, overlapping that of Fe²⁺.

Dissolved DRSi concentrations were approximately 2-fold higher at site D than at site U; over the course of the incubation values tended to augment in bare sediments and remained constant in vegetated sediments. Nitrate concentrations increased in the first half of the incubation period up to 40 μM, and then decreased to initial values of about 10 μM. Differences between sites and treatments were statistically not significant as well as relationships with macrophyte presence were not evidenced. To sum up, at both sites *V. spiralis* controlled the pore water concentrations of CH₄ and NH₄⁺ and promoted oxidised conditions, preventing PO₄³⁻ and Fe²⁺ accumulation within sediments. The patterns of pore water Eh, CO₂, PO₄³⁻ and Fe²⁺ in bare sediments were instead different between sampling sites, suggesting higher respiration rates and more reduced conditions downstream, in agreement with significantly higher organic matter content.

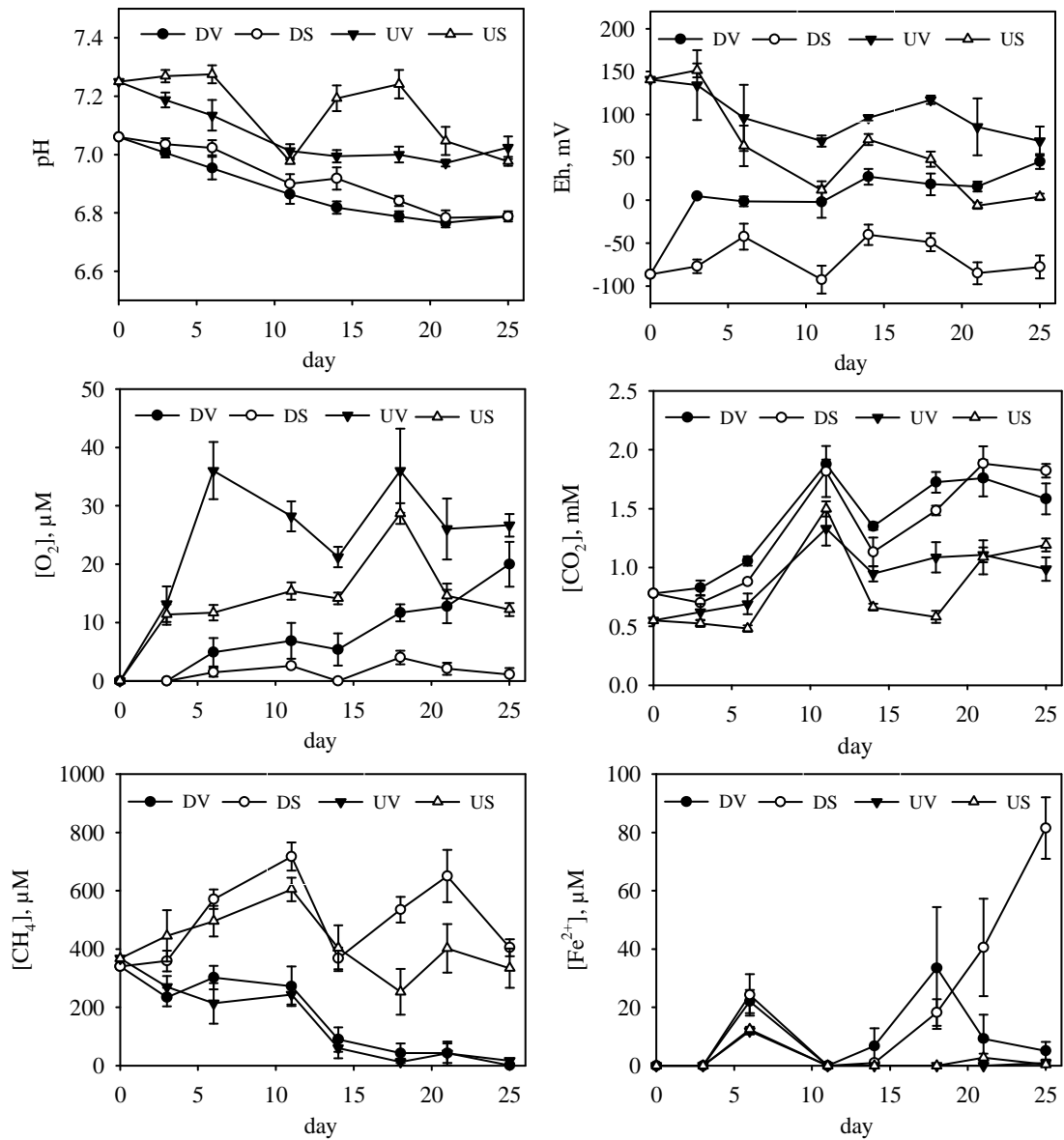


Figure 6.1. Course of pore water pH, CO₂, O₂, Eh, CH₄ and Fe²⁺ during the 25 days incubation period, from the 10th of March to the 4th of April 2009. Approximately every 4 days, 3 replicates for each experimental condition were analyzed. U_B=upstream bare sediment; U_V=upstream vegetated sediment by *V. spiralis*; D_B= downstream bare sediment, D_V=downstream vegetated sediment by *V. spiralis* (mean±SE, n=3).

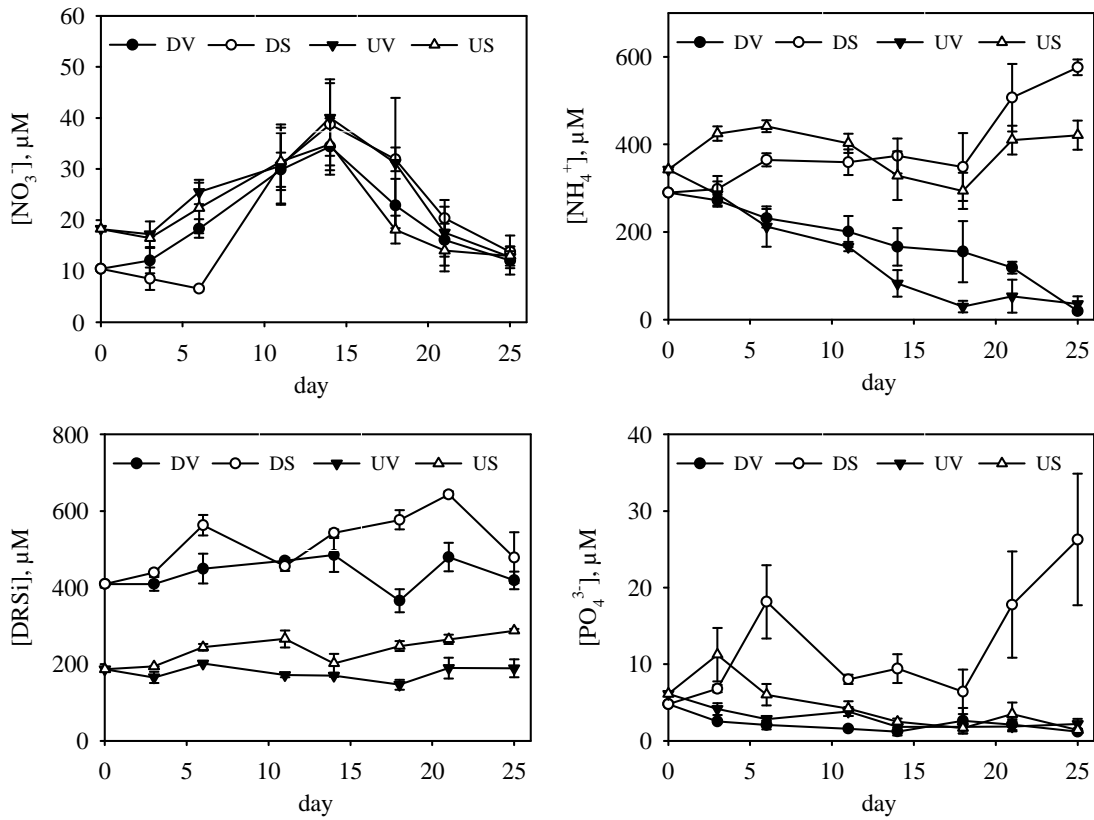


Figure 6.2. Course of pore water PO_4^{3-} , NH_4^+ , DRSi and NO_3^- concentrations during the 25 days incubation period, from the 10th of March to the 4th of April 2009. Approximately every 4 days, 3 replicates for each experimental condition were analysed. U_B =upstream bare sediment; U_V =upstream vegetated sediment by *V. spiralis*; D_B = downstream bare sediment, D_V =downstream vegetated sediment by *V. spiralis* (mean \pm SE, n=3).

Table 5. Levels of significance of contrasts analysis: vegetated versus bare sediment (V vs B); vegetated sediments upstream and downstream the sewage plant (U_V vs D_V) and bare sediments upstream and downstream the sewage plant (U_B vs D_B) are reported. Slopes compared in the contrasts analysis, calculated by means of ANCOVA, are reported in Table 4.

	V vs B	U_V vs D_V	U_B vs D_B
pH	n.s.	n.s.	n.s.
CO_2	n.s.	n.s.	<0.05
O_2	<0.01	n.s.	n.s.
Eh	<0.001	<0.001	<0.001
CH_4	<0.001	n.s.	<0.05
NH_4^+	<0.001	n.s.	<0.001
SiO_2	<0.01	n.s.	n.s.

6.4 Discussion of the results

6.4.1 ROL and redox dependent sediment features

In the Mincio River, *V. spiralis* colonises muddy substrates, where organic matter enrichment fuels intense respiration activities (Pinaridi et al., 2009). Under these circumstances, the survival of submerged macrophytes depends on their ability to transport oxygen to the rhizosphere, which allows roots respiration. ROL occurs with different mechanisms in a number of emergent, floating and submerged aquatic plants (Armstrong, 1971; Dacey, 1980; Sand-Jensen et al., 1982; Laskov et al., 2006; Soda et al., 2007). ROL from emergent or floating plants is generally greater than in submerged macrophytes, due to the high partial pressure of atmospheric oxygen (Dacey, 1980; Armstrong & Armstrong, 1988; Große, 1996). In submerged plants, ROL is generally a small fraction of the total photosynthetic oxygen, because in the underwater environment a great oxygen quota is used for respiration of roots and submerged tissues (Sand-Jensen & Prahl, 1982; Kemp et al., 1986).

To our knowledge, oxygen transport to roots and the evaluation of its effect on pore water chemistry was not previously measured for *V. spiralis*, although Pinaridi et al. (2009) already provided an indirect estimate of oxygen transport to the rhizosphere of *V. spiralis* from photosynthetic quotients. Oxygen released by roots to the rhizosphere cannot be evaluated from the presented data as most of it was immediately consumed by biotic and abiotic reactions. In our study, direct measurements of pore water oxygen demonstrated anyway that concentrations were significantly greater in vegetated compared to bare sediments, and CH₄ data suggest that part of the oxygen released by roots was actively used by microbial communities. In *V. spiralis* stands, a distinct clear sediment layer was evident all around root hairs, with a thickness of 1 to 2 mm, contrasting with the surrounding dark brown sediment (visual observations).

The oxygen concentration measured in the upper sediment layer (0-5 cm depth) at site D was lower than that at site U, probably because of the sedimentary organic enrichment generated by the sewage treatment facility. Here, organic matter decomposition caused hypoxia and negative redox potentials to establish mainly in bare sediments, where those conditions were accompanied by the release of Fe²⁺ and PO₄³⁻ in pore water, which is a typical feature of highly reduced sediments (Hejris et al., 2000; Azzoni et al., 2005). Overall, during the course of this experiment, carried out in the vegetative period of *V. spiralis*, oxygen concentration and redox potential were significantly greater in vegetated sediments, demonstrating that ROL from *V. spiralis* was able to keep oxic conditions in the rhizosphere.

ROL has a variety of direct and indirect effects on pore water among which the enhancement of the oxidation rates of CH₄ (Carpenter et al., 1983; Jespersen et al., 1998; Gerard &

Chanton, 1993; van der Nat & Middelburg, 1998; Heilman & Carlton, 2001), iron and manganese with formation of metal plaques on plant roots and parallel sequestration and precipitation of reactive phosphorous, mainly with iron hydroxides (Roden & Wetzell, 1996; Wigand et al., 1997). Wigand et al. (2000) demonstrated that *Vallisneria americana* Michaux had an elevated ROL which raised Eh values up to +125mV, allowing the formation of iron plaques on roots. Inorganic phosphorus was then bound by iron oxides and precipitated from pore water. Under oxic conditions, symbiotic associations between *V. americana* roots and fungi also facilitated phosphate uptake, giving a competitive advantage to *V. americana* (Wigand et al., 1997).

Results from our study are consistent with previous findings and indicate that the colonization of *V. spiralis* significantly affected sediment redox and related processes. In vegetated sediments, CH₄ concentrations was almost set to zero, as a consequence of CH₄ oxidation processes or competition with iron reduction processes (Roden & Wetzell, 1996; Neubauer et al., 2005). This is in agreement with findings reported by Boon & Sorrell (1991), which measured lower CH₄ fluxes in a *V. gigantea* meadow with respect to bare areas.

Effects of the ROL on the release of PO₄³⁻ and NH₄⁺ from pore water to the overlying water were also demonstrated for vegetated sediments at both sites, which differed for organic matter content. Previous investigations demonstrated that benthic vegetation can control sediment biogeochemistry only in oligotrophic systems, whereas under eutrophic conditions ROL was thought to be compensated by an elevated respiratory demand (Sand-Jensen & Borum, 1991; Hemminga, 1998). Results from the present study question such conclusions, as the effects of *V. spiralis* on pore water features seemed not to depend upon the organic content.

Studies on benthic biogeochemistry of silica are rather scarce and mainly addressed to the relationships with benthic diatoms (Sigmon & Cahoon, 1997), a few macrophyte species (Ma et al., 2001). In the present study, greater DR_{Si} concentrations in surface and pore water at site D were likely due to the wastewater plant and to decomposition of diatoms drifted from upstream and sedimented and could favour PO₄³⁻ mobilization from binding sites (Tallberg, 1999). Higher DR_{Si} pore water concentrations in unvegetated sediments at both sites could be due to redox dependent silica mobilization from particles (Michalopoulos & Aller, 1995) or to uptake by the plants. (Struyf & Conley, 2009).

6.4.2 Nitrogen speciation and rhizosphere processes

Ammonium, which is generally the dominant nitrogen form in pore water, is assimilated preferentially by aquatic macrophytes. However, in NO_3^- enriched waters, submerged vegetation can assimilate also NO_3^- from the canopy (Cedergreen & Madsen, 2003). *V. spiralis* shoots used in this experiment were collected at two sites differing for water column NO_3^- , with 4-fold higher concentrations at site D, and were incubated simulating the natural nitrogen availability. One of the hypotheses of this experiment was that in a flowing system with elevated NO_3^- supply in the water column leaf uptake would be stimulated, making the contribution of roots to pore water NH_4^+ uptake less important. On the contrary, during the course of the experiment, pore water NH_4^+ decreased to undetectable values, with no significant differences between U and D. This means that higher NO_3^- availability in the water column at site D did not affect NH_4^+ uptake in the rhizosphere. On the other hand, in vegetated sediments both ammonification and ammonium nitrification could be stimulated by aerobic conditions determined by ROL (Risgaard-Petersen & Jensen, 1997). In the present experiment, NO_3^- initially accumulated in pore water and then decreased without distinct patterns between vegetated and bare sediments and between sites. These results suggest that nitrification associated to the rhizosphere was not quantitatively relevant or that this process, if stimulated by ROL, was tightly coupled with denitrification. Ammonium decrease in pore water could be thus attributed to a combination of root uptake and/or denitrification coupled to nitrification; both processes are due to the presence of the macrophyte.

A comparison of the isotopic signature of plant tissues evidenced differences between U and D sites, which suggested a possible nitrogen uptake by the leaves. Wastewaters have isotopic signatures distinct from the atmospheric standard due to preferential volatilisation of $^{14}\text{NH}_3$ during wastewater processing. Therefore, NO_3^- resulting from oxidation processes presents a ^{15}N enrichment, on average ranging between +2 and +30‰ (Macko & Orstom, 1994). This could be the reason for elevated ^{15}N signature in *V. spiralis* biomass at site D, which can be due to the assimilation by the leaves of ^{15}N -enriched nitrate resulted from the treatment plant effluents. A significant ^{15}N enrichment was evidenced in the sediment particles at site D, which has been receiving urban effluents for more than twenty years. Therefore, the sediment itself can be a possible source of ^{15}N to *V. spiralis*. Furthermore, epiphytic microalgae could be a confounding factor, since they can assimilate ^{15}N from the water column, which is then accounted in the total nitrogen of the biomass bulk. At present, we do not have sufficient data to discriminate between assimilation from leaves and from roots. However, we can postulate that leaves uptake of nitrogen from the water column could also occur in a later stage of the

colonization process, when the nitrogen demand by macrophyte exceeds its availability in pore water.

Based on these results we can assume that NH_4^+ uptake by *V. spiralis* roots was the main cause for decreasing NH_4^+ concentrations in pore water. We can also speculate that, as reported for many other aquatic macrophytes, *V. spiralis* assimilated preferentially NH_4^+ even if exposed to elevated NO_3^- concentrations in the water column. Therefore, in the early stages of sediment colonization *V. spiralis* had a great potential to control NH_4^+ , thus attenuating its pore water content and possible delivery into the water column. *V. spiralis* preserved the capacity of controlling pore water processes also at site D, whose sediments had an elevated organic matter content and were a theoretically less favourable environment to colonize. Disputing previous investigations, which assumed that submerged vegetation is highly sensitive to eutrophication processes and thus to higher organic matter input to the benthic system (Nixon, 1995, Sand Jensen et al., 2008), our results suggest that *V. spiralis* has the potential to colonise organic matter impacted sediments, acting as an engineer species, which is able to modify and control pore water composition.

7. Influence of the aerenchyma of floating-leaved plants (*Nuphar luteum*) on C fixation-emission at the water-air interface

7.1 Aim of the study

Floating-leaved macrophytes have been studied thus far with special awareness to methane emissions, which are mediated by the pressurized ventilation inside the aerenchyma (Ohno, 1910; Dacey & Klug, 1979; Grosse & Schroder, 1984, 1986, 1987; Schroder, Grosse, and Woermann, 1986; Grosse and Mevi-Schultz, 1987). Methane concentration inside petioles can reach values up to 10 ml L⁻¹ and lead to emissions up to 13 mmol CH₄ m⁻²d⁻¹ (*Nuphar luteum*; Smith & Lewis, 1992; Yavitt & Knapp, 1998). Methane efflux from helophytes seems to be regulated by stomata opening other than pressurized ventilation, generating higher fluxes during the daylight compared to the dark (van der Nat & Middelburg, 1998; Käki et al., 2001), yet the diurnal patterns measured on floating-leaved plants remain still under discussion and seem to be dependent on a combination of factors such as air temperature and humidity (Yavitt & Knapp, 1998), other than the type of leaf (aerial or floating), which can be potentially heated or not by the water at night and sustain the flow (Dacey, 1981; Dacey & Klug, 1982).

Several studies have highlighted the relative responsibility of the riparian vegetation on GHG emissions: different authors report that methane fluxes measured on those areas are sensibly higher when compared to those measured on the open water (Kankaala *et al.*, 2005; Wang *et al.*, 2006). Most of those studies deal with emissions from helophytes (van der Nat & Middelburg, 1998; Brix *et al.*, 2001; Käki *et al.*, 2001; Kankaala *et al.*, 2005), while a few investigations still exist about methane efflux from floating-leaved rhizophytes (Smith & Lewis, 1992; Juutinen *et al.*, 2003; Kankaala *et al.*, 2003). Among those studies only one focuses on the carbon fixation (Larmola et al., 2003), even if this is a key issue when evaluating the influence that the primary producers have on net greenhouse gas emissions and radiative forcing. Floating-leaved rhizophytes can indeed affect the GHG emissions by direct and indirect effects, primarily the CO₂ uptake from the atmosphere and the CH₄ efflux from the aerenchyma, secondarily the oxidation processes in the rhizosphere favoured by pressurized ventilation and radial oxygen loss (ROL) (Grosse et al., 1996). The occurrence of the floating leaves can lead to the occlusion of the water surface with subsequent effects on the diffusion of the oxygen from the atmosphere and the accumulation of dissolved methane and carbon dioxide in the water column (Williams & Hecky, 2005; Bolpagni et al., 2007).

In this study I report carbon dioxide and methane measurements carried out on a *Nuphar luteum* stand together with seasonal biomass measurements; the overall aim was to evaluate the proportion of equivalent carbon released to the atmosphere compared to that fixed into biomass. Measurements with floating chambers were coupled to water biogeochemistry monitoring, with special regards to dissolved gases dynamics at the bottom and the surface of the water column. Those measurements were aimed at focusing on the influence of the oxygen transport from aerenchyma to the methane efflux from the sediment and to evidence potential carbon uptake from the water column. Data were compared to an open water site and related to environmental factors.

7.2 Sampling program and calculations

7.2.1 Incubations

From July 2008 to August 2009, carbon dioxide (CO₂) and methane (CH₄) fluxes across the water-air interface were measured in a yellow waterlily (*Nuphar luteum*) stand by means of plexiglass floating chambers. Measurements were performed in a 0.2 ha pond during the different phases of the vegetative period of the plant, namely the time of germination (May 2009), the biomass peak (July and August 2008, August 2009) and the senescence phase (September 2008). Prior to the start of the experiment, a simple docking formed by three poles was placed within the *N. luteum* stand, in order to make the boat positioning in the same way at every campaign and incubation. The same care was taken on a nearby station with open water surface in the same pond.

For each sampling campaign, a 24h cycle was carried out consisting of short-term incubations repeated approximately every 2-3 hours. Within each incubation, two floating chambers were laid from the boat upon two different stands of *N. luteum* leaves, and another one was laid upon the surface of open water as control. Each chamber was made of plexiglass and had a surface area of 80×80 cm and a inner volume of 0.128 m³; the interior was equipped with two fans and with an airbag connected to the exterior by a tube in order to avoid underpressure in the chamber headspace. The chambers laying on the plants enclosed from 8 to 12 floating leaves each; the setting of the dock permitted to incubate approximately the same leaves, whose number was recorded at each measurement. Gas samples were collected at 0, 3, 6 and 10 minutes by means of a plastic syringe (60 ml) connected to a plastic tube and carefully flushed in a gas-tight vial (Exetainer Labco, High Wycombe, UK; volume 12 ml) (Figure 7.1). Samples were kept in a refrigerated box until analyses in

laboratory. Variations in PAR intensity were monitored every 20 minutes all day long by means of a luxometer (Delta OHM, HD9021 model); measurements were intensified during incubations (every 3 minutes).

7.2.2 Water sampling and analysis

Prior to the start of the experiment, two plastic tubes were positioned within the plants mat and fixed on a pole at two different level of the water column, few millimeters above the bottom and under the water surface, respectively. The same operation was carried out on the open water site. The collection tubes were 3 m long and far from the boat docking, in order to avoid any disturbance due to boat movements. In correspondence with every incubation, water samples were collected from the surface and from the bottom by means of plastic syringes; the water column was 70 cm and 100 cm deep at the vegetated site and in the open water, respectively. Water temperature and pH were recorded immediately; water samples were treated and stored for dissolved gases (O₂, CO₂ and CH₄), DIC and Chl-*a* measurements as described in chapter 4.

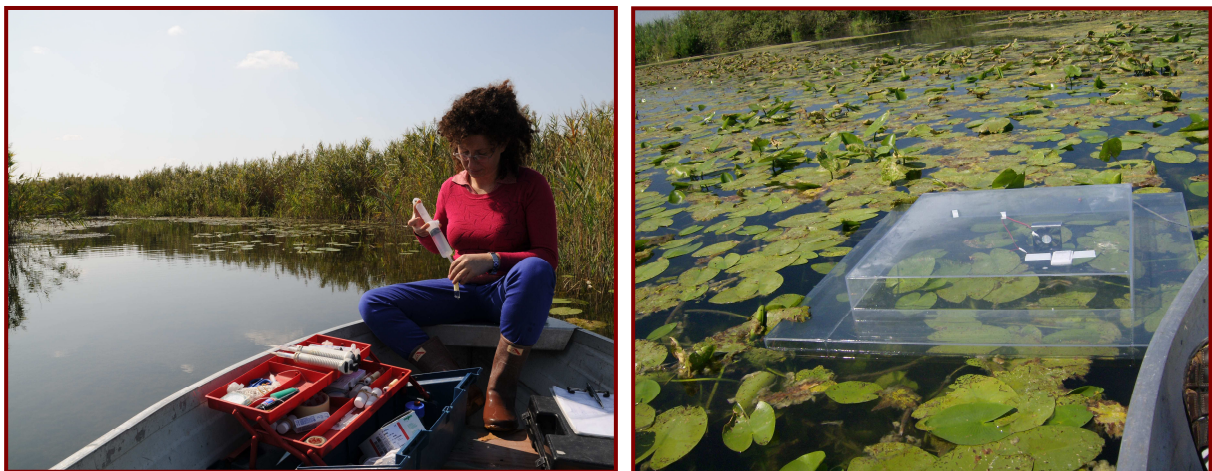


Figure 7.1. Water sampling (on the left) and placement of a floating chamber on a *N. luteum* stand (on the right) during May 2008.

7.2.3 Plants measurements

During the campaigns of August 2008, May and August 2009, *N. luteum* shoots (floating leaves + submerged leaves + petioles) were harvested in three replicates for biomass measurements (frame area= 1 m²). Shoots were kept in tanks filled with water and transported to the laboratory, where they were rinsed with tap water and separated into leaves and petioles. Shoot density (shoots m⁻²) was recorded (floating leaves + submerged leaves);

leaf surface ($\text{cm}^2 \text{ leaf}^{-1}$) was calculated as $(\text{length} \times \text{width})/2$. For each replicate, five leaves were dried and weighed separately at 70°C for 72 hours for Specific Leaf Weight determination (SLW, $\text{mg}_{\text{dw}} \text{ cm}^{-2}$). Leaf Area Index (LAI, $\text{m}^2 \text{ m}^{-2}$) was calculated by multiplying the average leaf surface for the average number of leaves in a square meter (floating + submerged leaves). Shoot density and LAI referring to the campaign of July and September 2008 were calculated from the number of leaves incubated during each incubation. Petioles were measured from the basis to the blade (cm) and the cross-section surface (cm^2) was measured at the basis and at the apex; ten replicates of 5 cm long petiole segments were dried and weighed after 72 hours at 70°C . The mean weight of one shoot (leaf+petiole, g_{dw}) was determined by the sum of the average leaf and petiole weight. The shoots which were not used in morphometrics were dried together and weighted for total areal biomass determination ($\text{g}_{\text{dw}} \text{ m}^{-2}$). Biomass values referring to the campaign of July and September 2008 were calculated from the number of leaves incubated during each incubation multiplied for the mean weight of one shoot.

7.2.4 Water-air fluxes and biomass turnover

Water-air fluxes of CO_2 and CH_4 were calculated with a linear regression of concentrations *versus* incubation time, and expressed as hourly rates per square meter ($\text{mmol m}^{-2}\text{h}^{-1}$). Regressions were accepted as reliable when having a $R^2 \geq 0.50$, as the incubations carried out at the sunrise or the sunset were characterized by very low slopes due to mixed effects of different leaves. Positive fluxes are to be intended as directed from the water/leaves to the air and *vice versa*. Light fluxes ($\text{mmol m}^{-2}\text{d}^{-1}$) were calculated as the hourly fluxes measured during daylight ($\text{PAR} > 100 \mu\text{E m}^{-2}\text{s}^{-1}$) multiplied by the correspondent hours between incubations and then summed; dark fluxes ($\text{mmol m}^{-2}\text{d}^{-1}$) values refer instead to hourly fluxes summed during the dark hours. Daily fluxes ($\text{mmol m}^{-2}\text{d}^{-1}$) were obtained from the sum of light and dark fluxes. The July 2008 campaign being carried out only on the vegetated site during the daylight, our data lacked in control comparison and in dark fluxes. With the purpose of obtaining daily values also for this period, we assumed dark flux values equal to those measured during the following campaign in August 2008. All rates were converted to carbon dioxide equivalents ($\text{g CO}_2\text{-eq m}^{-2}\text{d}^{-1}$) accordingly to the GWP (Global Warming Potential) values reported by IPCC (2001) of 1 and 25, for CO_2 and CH_4 respectively. The GHG emission budget was then assessed for both sites by summing the contribution of the two greenhouse gases in terms of grams of CO_2 equivalents.

Net carbon fixation from *N. luteum* was calculated as the shoots production during the vegetative period, taking into account the period May-August (120 days) and excluding the

month of September, when the leaves were visibly in a decline phase. Values were calculated according to the formula:

$$NS = \left(SD + \frac{t}{LLS} \right) \times \frac{1}{LA} \times t$$

where NS are the number of shoots (shoots m⁻²) produced during the period t (d), SD is the shoot density (shoots m⁻²) for each campaign, LA is the leaf appearance rate (d) of 15 days (Kouki, 1991; Titus & Sullivan, 2001) and LLS is the average leaf life span or leaf longevity (d) of 35 days (Twilley et al., 1985; Wallace & O'Hop, 1985; Kouki, 1991; Setälä & Mäkelä, 1991). The length of the period for each campaign was assumed to be of 90 days for July 2008 (extended for May to July), of 30 days for August 2008, of 60 days for May 2009 (for May to June) and of 60 days for August 2009 (for July to August). The number of shoots obtained was then multiplied by the shoot mean weight (g_{dw}) and a carbon content in the vegetal tissue of 0.38 g C g_{dw}⁻¹ (Longhi et al., 2008) and expressed as g C m⁻². Values from each campaign were summed to obtain an annual budget for 2008 and 2009. Those values were compared to net carbon fixation estimates obtained by daily CO₂ fluxes measured on *N. luteum* and extended to each correspondent sampling period.

7.2.5 Statistical analyses

Differences in water temperature and chlorophyll concentration were tested by means of ANOVA and Tukey's HSD multiple comparison test, with sites and seasons as factors. Prior to the analyses, all CH₄ values were log-transformed for normality requirements. Differences in dissolved gases concentration, pH values and DIC concentration were tested with site, season and level as factors. Water-air flux comparisons between sites couldn't be tested on the single incubations due to the lack of a replicate on the control site. The two sites were thus compared for each campaign in average hourly rates by means of the Student's *t* test. Seasonal and daily patterns were tested on each site separately, by considering each incubation as a replicate; differences were tested by ANOVA and Tukey's HSD. Linear regressions were carried out to test dependence of dissolved gases and of CO₂ and CH₄ fluxes from water temperature and irradiance. Analyses were performed using the R Program (R - Development Core Team, 2009); statistical significance was set at p≤0.05. All average values are reported with associated standard error (SE).

7.3 Results

7.3.1 General features of the water column and of *N. luteum* plants

During the spring and summer incubations, *N. luteum* leaves looked green and healthy, yet in September the stand was formed only by old leaves, mostly damaged by the gnawing of the water-lily beetle *Galerucella nymphaeae*. Number of floating leaves incubated at each incubation, shoot densities, biomass and LAI values are reported in Table 1. Average number of submerged leaves was 2.1 ± 0.2 , 3.4 ± 0.6 and 1.9 ± 0.9 m^{-2} , for August 2008, May and August 2009, respectively. Average leaf surface was of 357.4 ± 25.3 cm^2 leaf⁻¹, while SLW was of 16.7 ± 0.7 mg_{dw} cm^{-2} . Average petiole length was of 93.4 ± 5.5 cm, with a mean cross-section surface of 0.15 ± 0.01 cm^2 . The mean weight of a shoot (leaf+petiole) was 6.93 ± 0.43 g_{dw} .

Table 1. Number of incubated leaves (floating leaves only) (mean \pm SE, n=12-18), biomass (mean \pm SE, n=3), shoot density (floating+submerged leaves) (mean \pm SE, n=3) and leaf area index (LAI, floating+submerged leaves) (mean \pm SE, n=3). * values were obtained from number of leaves incubated during each incubation and thus not include the submerged leaves (mean \pm SE, n=12-18).

Campaign	Number of incubated leaves floating leaves m^{-2}	Biomass g_{dw} m^{-2}	Shoot density shoots m^{-2}	LAI m^2 m^{-2}
07/16/2008	25.4 ± 0.7	$176.4 \pm 4.6^*$	-	$0.91 \pm 0.06^*$
08/06/2008	27.3 ± 0.5	203.4 ± 4.2	29.3 ± 0.3	1.05 ± 0.08
09/25/2008	10.3 ± 0.3	$71.2 \pm 2.2^*$	-	$0.37 \pm 0.03^*$
05/23/2009	11.6 ± 0.4	100.9 ± 2.8	14.5 ± 0.3	0.52 ± 0.03
08/01/2009	30.5 ± 1.0	230.5 ± 9.3	33.3 ± 1.3	1.19 ± 0.08

No significant difference occurred in water temperature according to the site or to the level of the water column. Water temperature showed higher values during summer (ANOVA, $p < 0.01$; Tukey's HSD, $p < 0.01$); daily values were comprised between 17.0 ± 0.3 (September 2008) and 29.9 ± 0.5 °C (August 2008) with peaks reaching 33°C (Table 2). pH values were higher at the vegetated site and at the surface level at all samplings (ANOVA, $p < 0.01$; Tukey's HSD, $p < 0.01$), with average daily values comprised between 7.61 ± 0.07 (bottom) and 7.99 ± 0.07 (surface), while on the open water values ranged from 7.57 ± 0.04 (bottom) to 7.96 ± 0.07 (surface). DIC values changed seasonally with higher values in May 2009 (ANOVA, $p < 0.01$; Tukey's HSD, $p < 0.01$) and showed no differences between sites and levels; average daily values were comprised between 3.07 ± 0.13 and 4.41 ± 0.14 mM DIC. Chl-*a* concentration changed seasonally (ANOVA, $p < 0.01$; Tukey's HSD, $p < 0.01$) and was

comprised between 2.4 ± 2.0 (September 2008) and $279.2\pm 5.8 \mu\text{g L}^{-1}$ (August 2009) with no differences between sites (Table 2).

Table 2. Water temperature (surface+bottom level, n=12-18; July 2008 measurements refer to surface only, n=8) and chlorophyll concentration (n=3) measured in the water column of the two sites (mean \pm SE).

		Site	
		Open water	<i>N. luteum</i>
Water temperature (°C)	Campaign		
	July 2008	25.3 \pm 0.7	26.1 \pm 0.8
	August 2008	29.9 \pm 0.7	28.9 \pm 0.8
	September 2008	17.0 \pm 0.6	17.2 \pm 0.8
	May 2009	26.9 \pm 0.8	27.6 \pm 1.0
	August 2009	29.3 \pm 0.7	29.1 \pm 0.8
Chl-a ($\mu\text{g L}^{-1}$)	July 2008	17.1 \pm 1.0	30.9 \pm 1.0
	August 2008	15.7 \pm 5.4	20.6 \pm 3.4
	September 2008	5.4 \pm 2.0	2.4 \pm 2.0
	May 2009	31.4 \pm 2.9	27.3 \pm 1.4
	August 2009	279.2 \pm 5.8	251.4 \pm 12.9

Gas saturation at the two levels of the water column at the two sites are reported in figure 7.1, 7.2 and 7.3. Superficial water at both sites was characterized by an O₂ deficit during the night and early morning and by supersaturation after noon at every campaign except for the one of September, when values were always below 100% (Figure 7.1). Bottom water was generally oxygen deficient at every campaign at every site; differences between levels were not dependent from the site (ANOVA, p<0.001). The overall O₂ concentration values were between 0.01 and 0.35 mM.

The water column was constantly CO₂ supersaturated, with higher values at the bottom level at every campaign (ANOVA, p<0.001; Tukey's HSD, p<0.01), and no differences between sites (Figure 7.2). Values at the surface level followed a daily pattern with lower values during the afternoon and higher values during night and early morning. Overall CO₂ concentration values were between 0.02 and 0.41 mM. At both sites, the water column was supersaturated in methane at every sampling (Figure 7.3). Values differed significantly between sites at the surface level, with higher values at the *N. luteum* site (ANOVA, p<0.005; Tukey's HSD, p<0.005); at this site, values at the bottom level followed a slight decline along the day. Bottom values at the open water site were generally higher than the

surface ones at every campaign (ANOVA, $p < 0.005$; Tukey's HSD, $p < 0.05$), with a peak during the early morning hours. Methane seasonal shifts were attributable to water temperature fluctuations, with higher fluxes at higher temperatures ($R^2 = 0.27$, $p < 0.001$, $n = 90$): overall CH_4 concentration values at the two sites varied between 0.08 and 9.62 μM .

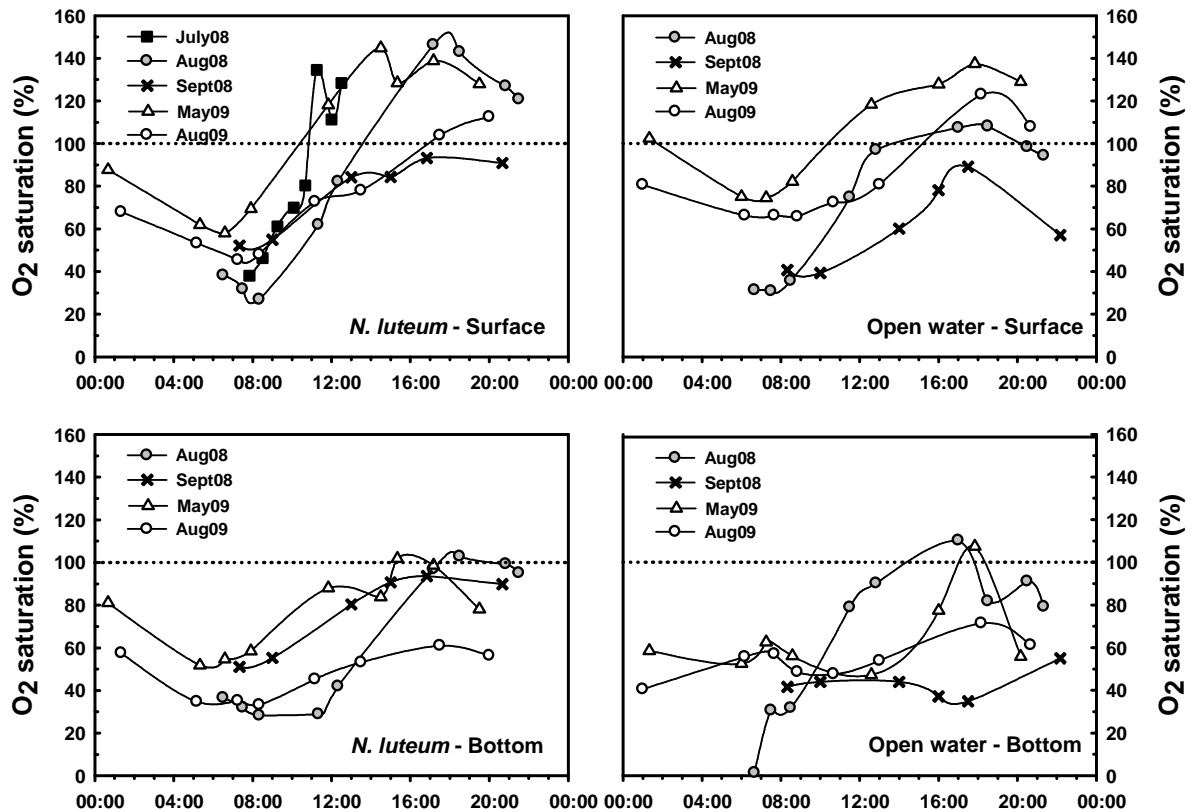


Figure 7.1. Oxygen saturation (%) measured along the day in a *N. luteum* stand and on the open water at two different levels of the water column (surface and bottom). The dotted line indicates the 100% of saturation, i.e. the theoretical concentration the gas should have at a given temperature if in equilibrium with the atmosphere.

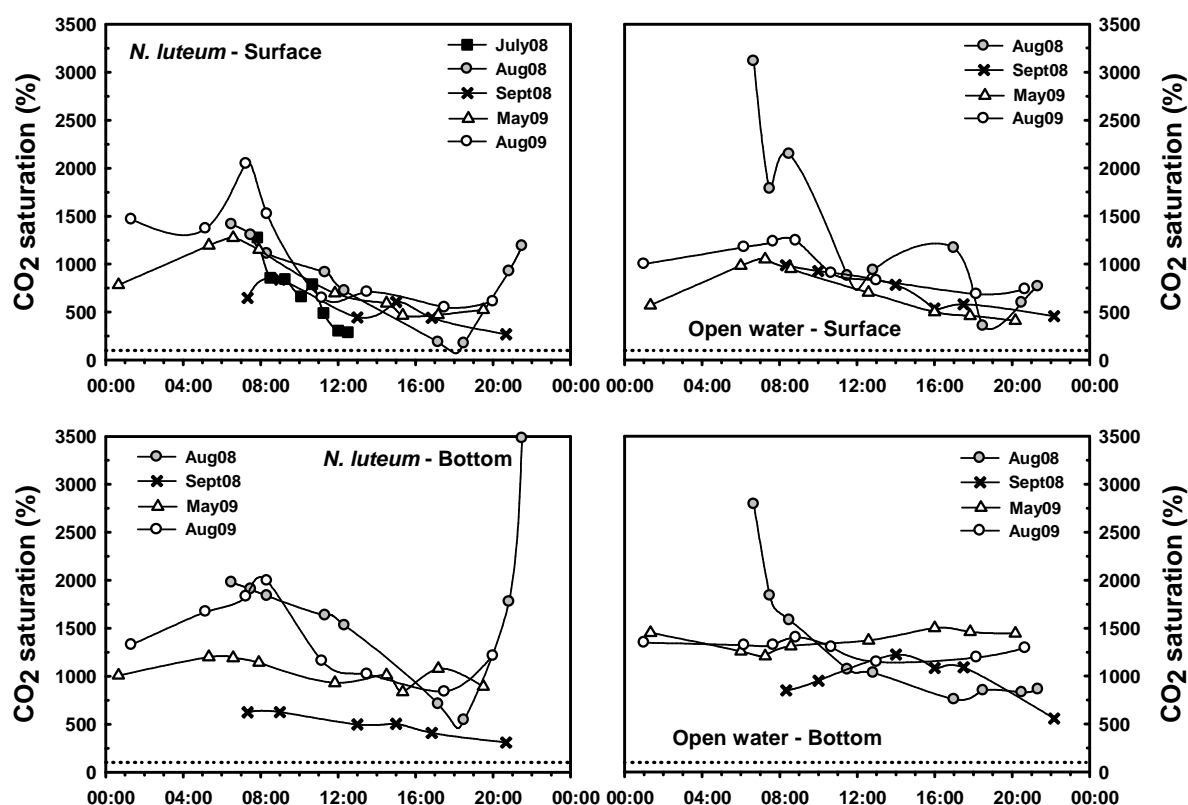


Figure 7.2. Carbon dioxide saturation (%) measured along the day in a *N. luteum* stand and on the open water at two different levels of the water column (surface and bottom). The dotted line indicates the 100% of saturation, i.e. the theoretical concentration the gas should have at a given temperature if in equilibrium with the atmosphere.

7.3.2 Fluxes across the water-air interface

Carbon dioxide fluxes measured at the water-air interface differed significantly between sites (Student's *t*, $p < 0.05$ at every comparisons). Hourly CO₂ fluxes measured on the open water were between -2.20 and 24.57 mmol m⁻²h⁻¹ (August 2009) (Figure 7.4), while on the *N. luteum* stand they were between -17.9 ± 4.7 and 10.8 ± 0.1 mmol m⁻²h⁻¹ (May and August 2009, respectively) (Figure 7.4 and 7.5). CO₂ fluxes measured on *N. luteum* showed differences between light and dark values, the former indicating a net CO₂ uptake, except in September 2008, and the latter indicating a net release at every campaign (Figure 7.6). Light fluxes at the vegetated site varied between -134.0 ± 26.6 and 8.0 ± 13.9 mmol CO₂ m⁻²d⁻¹, while dark fluxes ranged from 11.1 ± 24.4 to 67.4 ± 35.0 mmol CO₂ m⁻²d⁻¹, measured in May 2009 and September 2008, respectively. Daily CO₂ fluxes were between -122.9 ± 2.2 and 75.4 ± 1.8 mmol CO₂ m⁻²d⁻¹, measured in May 2009 and September 2008. Open water behaved as a net CO₂ source at every sampling, with no apparent seasonal changes and daily fluxes between

108.6 and 119.4 mmol CO₂ m⁻²d⁻¹ (Figure 7.6). Light fluxes measured on the open water ranged between 39.2 and 97.6 mmol CO₂ m⁻²d⁻¹, measured in August and September 2008, respectively, while dark fluxes were between 18.5 and 80.2 mmol CO₂ m⁻²d⁻¹, measured in September and August 2008, respectively.

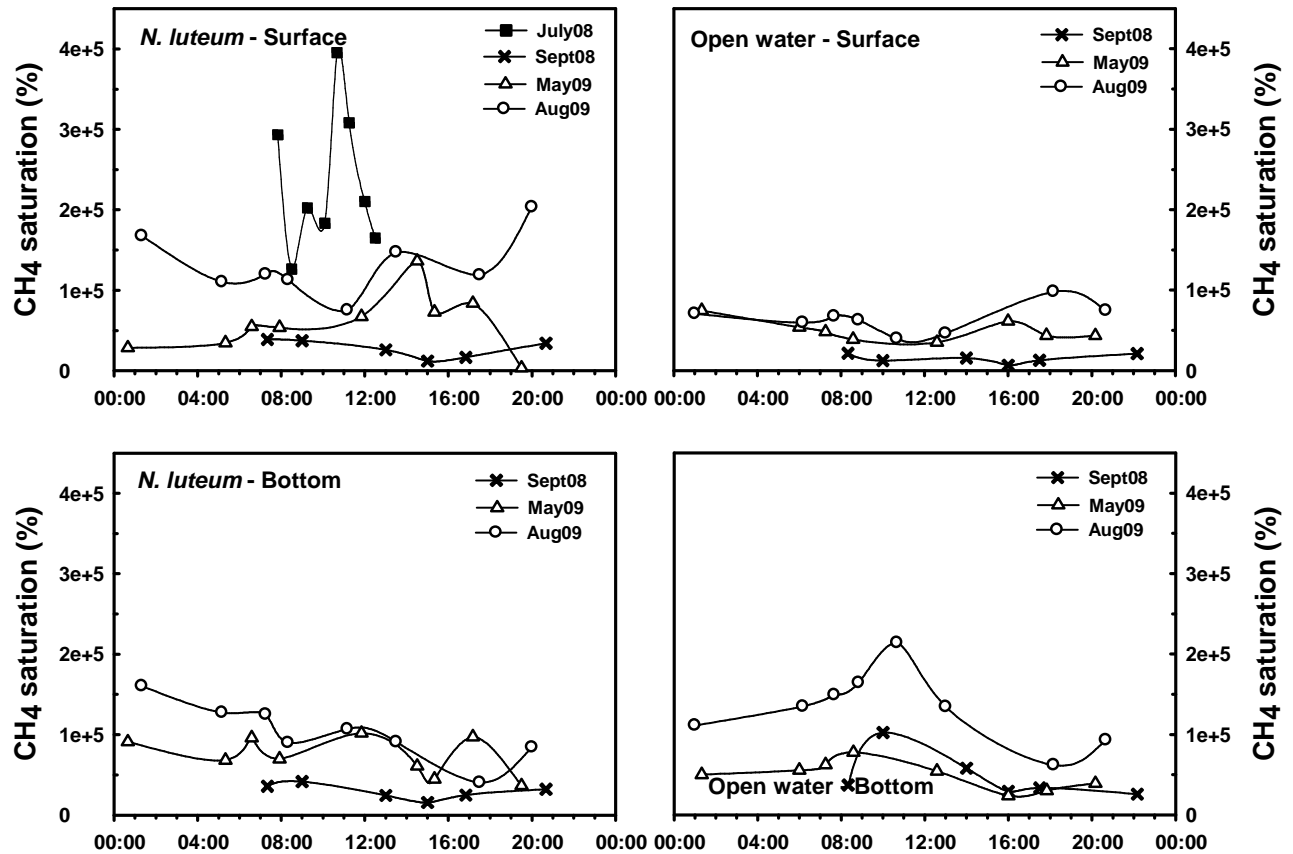


Figure 7.3. Methane saturation (%) measured along the day in a *N. luteum* stand and on the open water at two different levels of the water column (surface and bottom).

Methane fluxes measured at the two sites showed similar daily patterns and values, and both sites behaved as net sources of methane towards the atmosphere (Figure 7.5). Hourly CH₄ emission rates from open water were between 0.2 and 22.3 mmol m⁻²h⁻¹ (May and August 2009, respectively), while they ranged from 0.2±0.1 to 8.4±0.7 mmol m⁻²h⁻¹ on the *N. luteum* site (September and July 2008, respectively). At the *N. luteum* site, daily CH₄ fluxes followed a seasonal pattern, with a minimum of 9.1±0.5 (September 2008) and a peak of 94.0±0.2 mmol m⁻²d⁻¹ (August 2009) (ANOVA, p<0.01; Tukey's HSD, p<0.01) and were slightly higher than those measured on the open water, except for the campaign of

September. Light and dark CH₄ fluxes measured on *N. luteum* were comparable, with values going from 3.0±0.9 (Light, September 2008) to 52.2±4.2 mmol m⁻²d⁻¹ (Dark, August 2009) as well as those measured on the open water surface, which were between 3.9 (Dark, May 2009) and 38.9 mmol m⁻²d⁻¹ (Light, August 2009).

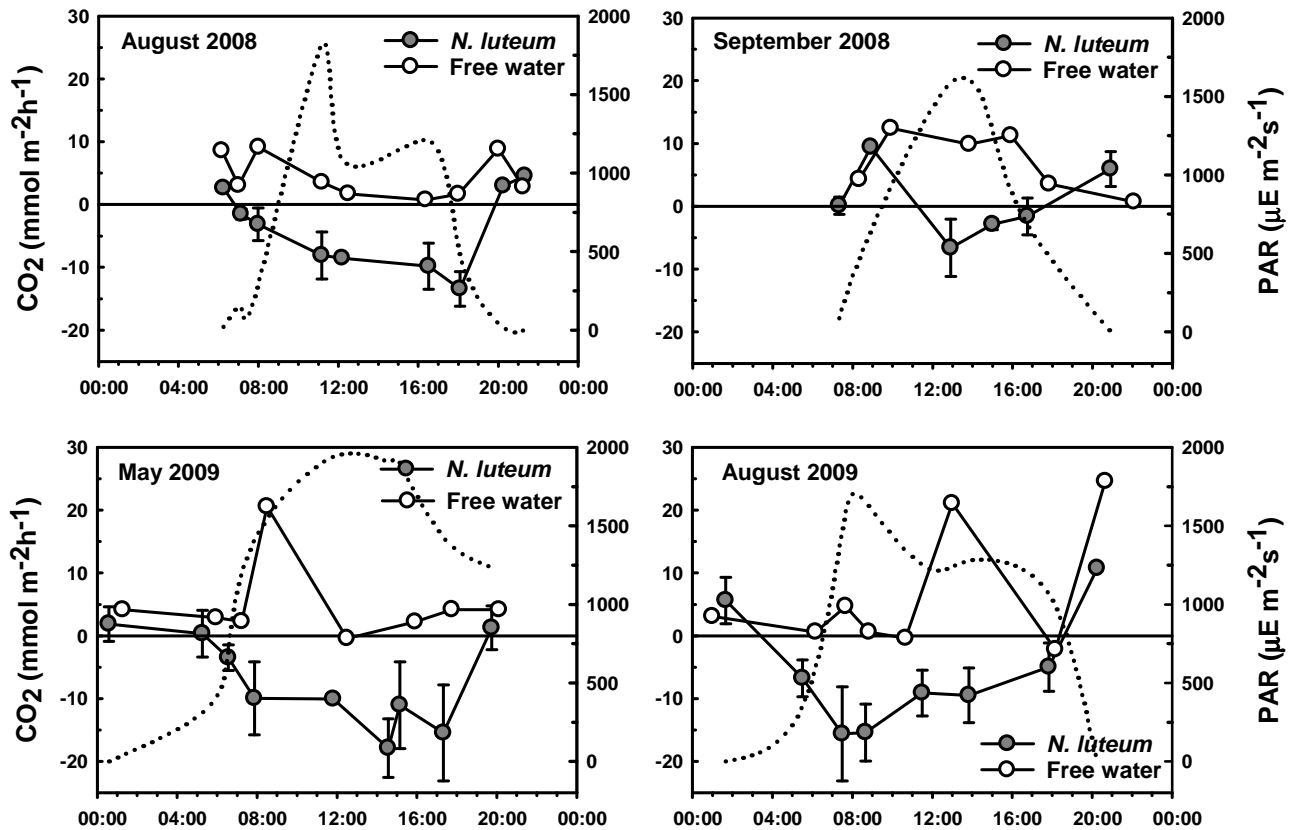


Figure 7.4. Hourly CO₂ fluxes measured on a *N. luteum* stand (n=2, mean±SE), and on a open water surface (n=1). Irradiance (PAR) is reported on the dotted line and refers to measurements recorded every 20 minutes all day long and every 3 minutes during incubations.

Methane fluxes were not correlated with PAR intensity, yet they were correlated with water temperature at both sites ($R^2=0.22$, $p<0.001$, $n=110$); carbon dioxide fluxes on the open water were not correlated to the irradiance values nor to the water temperature, except in September, when fluxes increased during daylight due to raising of water temperature (light \times temperature, Tukey's HSD, $p<0.01$). At the *N. luteum* site, carbon dioxide rates were strictly dependent on irradiance fluctuations ($R^2=0.49$, $p<0.001$, $n=79$), yet were not influenced by the water temperature.

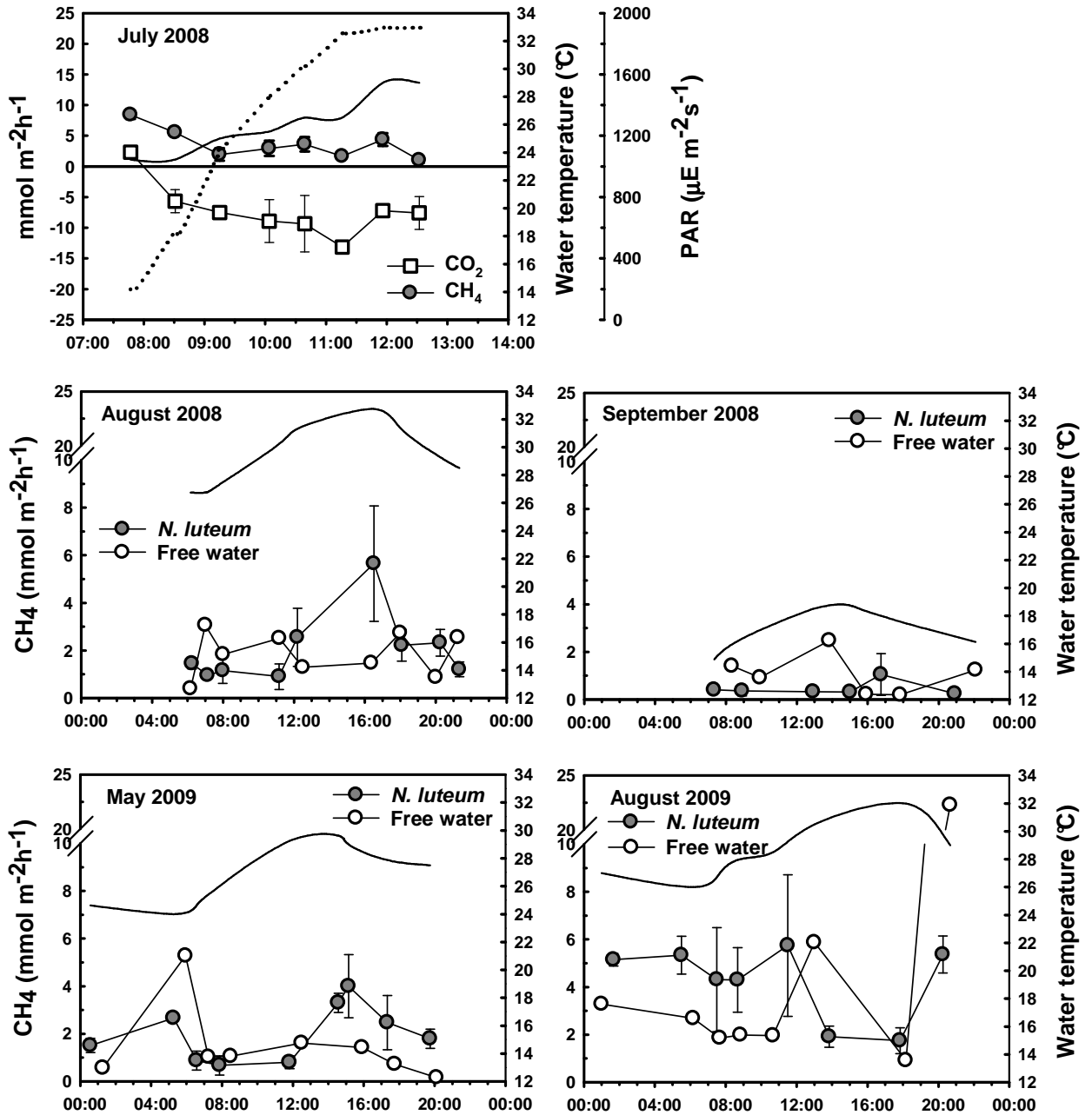


Figure 7.5. Top graph. Hourly CO₂ and CH₄ fluxes measured on a *N. luteum* stand (n=2, mean±SE), during daylight. Irradiance (PAR) is reported on the dotted line, temperature is represented as a continuous line and refers to measurements taken in correspondence of every incubation at the water surface. Bottom graphs. Hourly CH₄ fluxes measured on a *N. luteum* stand (n=2, mean±SE), and on a open water surface (n=1). Temperature is represented as a continuous line and refers to averaged values from measurements taken in correspondence of every incubation at the bottom and at the surface levels at both sites (n=4).

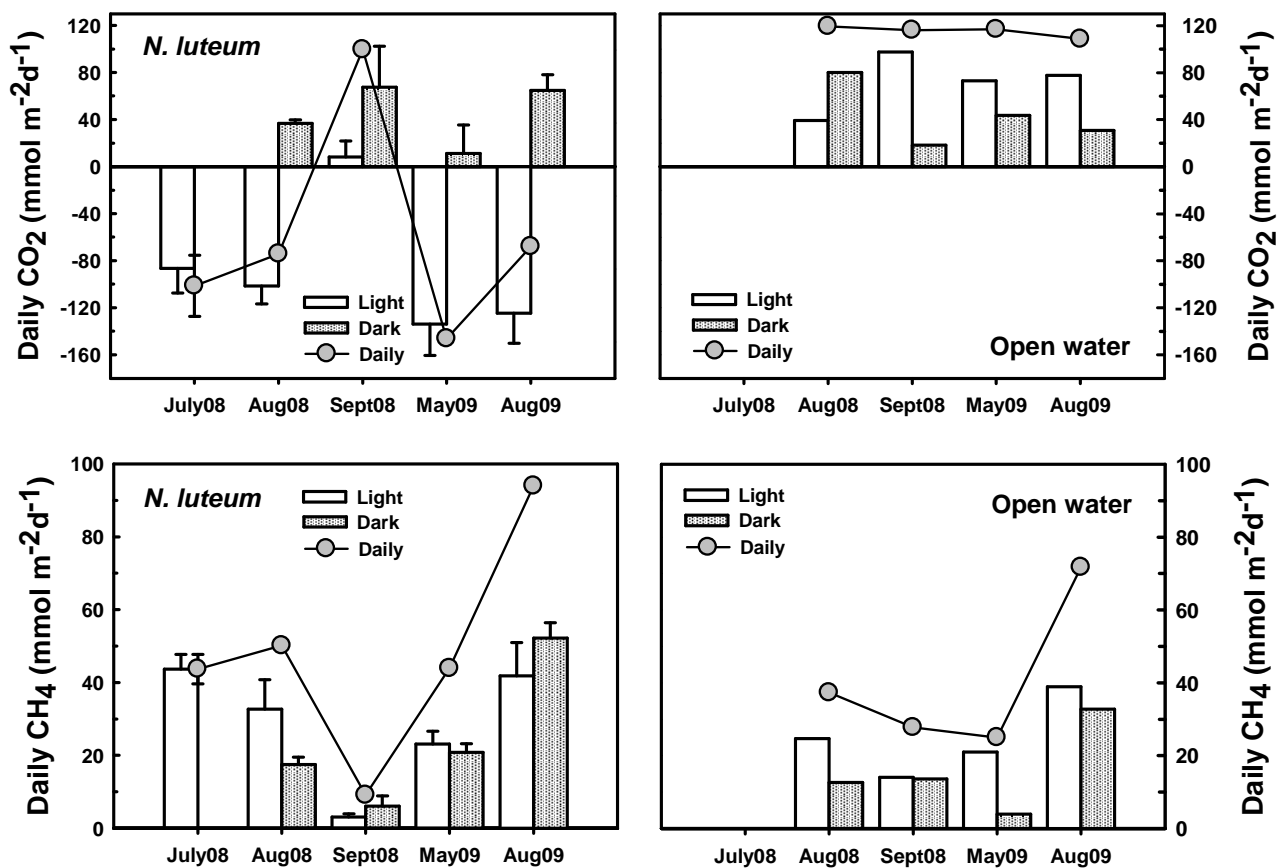


Figure 7.6. Light (light bars), dark (shaded bars) and daily (line and scatters) CO₂ and CH₄ fluxes measured on a *N. luteum* stand (left side) and on open water (right side). Daily values referred to July 2008 on the vegetated site were obtained by assuming a dark flux equal to that measured during the followed campaign. Note the different scales between CO₂ and CH₄ fluxes representations.

7.3.3 Greenhouse gas emissions and carbon fixation into biomass

Both vegetated site and open water behaved as a net source of greenhouse gases towards the atmosphere at every sampling campaign. When converted to carbon dioxide equivalents, the *N. luteum* emitted a minor or an equal amount with respect to the open water (Figure 7.7); values ranged from 1.74 ± 0.03 to 7.90 ± 0.01 g CO₂-eq m⁻²d⁻¹ emitted from the *N. luteum* stand and from 3.69 to 7.87 g CO₂-eq m⁻²d⁻¹ emitted from the open water.

New shoots produced during the vegetative phase of *N. luteum* resulted in 229 ± 20 and in 205 ± 15 NS m⁻² for 2008 and the 2009, respectively. Those values are equivalent to a net carbon fixation into biomass of 602.0 ± 51.7 and of 539.8 ± 39.0 g C m⁻², for 2008 and 2009, respectively. Values obtained from CO₂ fluxes were 77.2 ± 22.9 and 131.5 ± 1.6 g C m⁻² for 2008 and 2009, respectively.

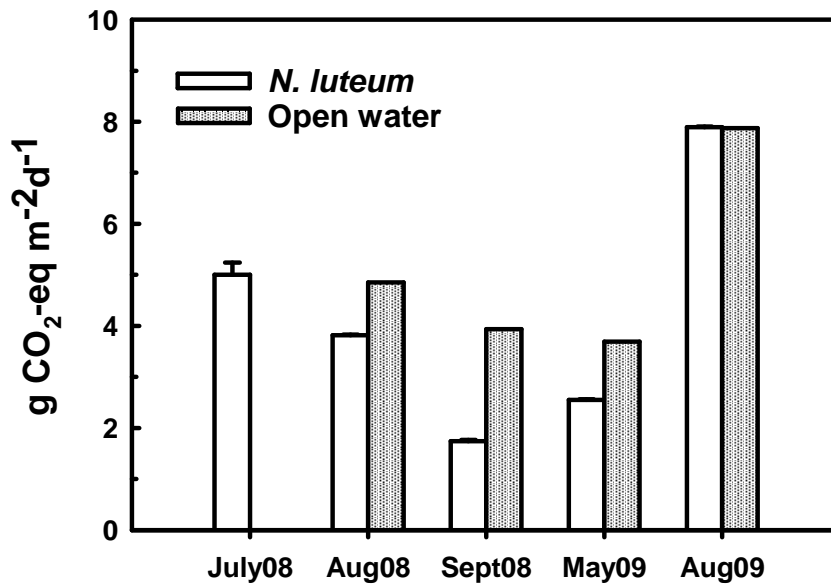


Figure 7.7. Greenhouse gas emissions from a *N. luteum* stand (n=2, mean±SE) and from the open water (n=1) of a shallow pond in the Northern Italy.

7.4 Discussion of the results

Floating-leaved macrophytes are seen as a natural channel connecting the sediment and the air and favouring the methane emissions towards the atmosphere. In the present study the methane and carbon dioxide emissions from a *N. luteum* stand were measured as well as the dissolved gas dynamics within the water column. The measured hourly methane fluxes were of the same order of magnitude as those reported by Dacey & Klug (1979), Dacey (1981) and by Smith & Lewis (1992), yet they were much higher than those reported by Kankaala et al. (2003, 2004) for a stand located in southern Finland, probably due to differences in water temperature and thus rates of microbial processes. The same can be stated for the methane fluxes measured on the open water, which were of the same order of magnitude as those reported by Delaune et al. (1983) and by Smith and Lewis (1992) yet higher than those measured by Chang and Yang (2003). Methane efflux from yellow waterlily was comparable or lower than values reported for *Phragmites* (Brix et al. 1996), and *Typha* sp. (Sebacher et al. 1985; Chanton et al. 1993). Both light and dark carbon dioxide fluxes were one order of magnitude higher than those reported by Larmola et al. (2003) on a *N. luteum* stand in central eastern Finland, probably due to higher activity by the plants favoured by warmer air and water.

The results from the incubations showed that the vegetated site substantially contributed to the CO₂ uptake from the atmosphere but also released a large amount of methane through the aerenchyma transport. As in the open water, the seasonality of daily CH₄ emissions from the yellow waterlily was evident, and the extent probably associated also to the vegetative phase of the yellow waterlily and shoot density within the stand, other than to water temperature. Moreover, methane fluxes measured on *N. luteum* didn't show any clear diurnal pattern, and no differences between light and dark fluxes were evidenced. Those results are in agreement with findings by Dacey and Klug (1982) which showed the persistence of the ventilation during the nighttime and differ from findings of Yavitt and Knapp (1998). The presence of the plants turned the system autotrophic at all samplings except during the fall season, when plants stopped active growth, while the open water continuously released both CO₂ and CH₄ to the atmosphere. The higher methane emissions from vegetated site did not lead to a greater total emission of GHG to the atmosphere due to CO₂ uptake into biomass by *N. luteum*. The biomass value we measured during summer was much higher than the maximum standing crop reported by Kankaala et al. (2003) and in the range of values reported in the Tsuchiya review (1991). The shoot density and the net carbon fixation into biomass were higher than those reported by Wallace & O'Hop (1985), Setälä & Mäkelä (1991) and Larmola et al. (2003). The leaf areas are in agreement with those reported by Dacey (1981), Wallace & O'Hop (1985) and Setälä & Mäkelä (1991), as was the leaf area index (Tsuchiya and references therein, 1991). These values are likely characteristic of this plant typology, which occupies the entire water surface during maximum standing crop.

The difference between the estimated carbon fixation from the CO₂ fluxes and from the aboveground biomass turnover, also reported by Larmola et al., (2003), supports the occurrence of another carbon source other than the atmospheric CO₂. Dacey (1980) and Dacey and Klug (1982a) already reported that up to 85% of the CO₂ produced in sediments or root tissues and moving to the shoots can be fixed via photosynthesis, and thus can constitute another carbon source. On the other hand, Smits et al. (1988) put in evidence the fact that nymphaeid water plants does not depend only on the gas phase for their carbon source because of their heterophylly. They evidenced that while the carbon uptake from the adaxial side of the floating leaves derives from the atmospheric CO₂, the growth of submerged leaves is completely dependent on the availability of dissolved inorganic carbon in water. The authors demonstrated that *N. luteum*, as other nymphaeid plants such as *Nymphaea alba* and *Nymphoides peltata*, uses dissolved CO₂ as primary source of carbon, when growing in well-buffered waters. This result is in agreement with the slight decline in CO₂ concentration we observed during the afternoon hours at the surface level, which

indicates a probable uptake by the submerged leaves. The underwater phase of the plant can thus play a major role in the carbon uptake of the plants, thanks to the fast time of renewal, often stimulated by herbivory on floating leaves (Wallace & O'Hop, 1985; Kouki, 1991; Setälä & Mäkelä, 1991). In general, abiotic water parameters did not differ between the two sites probably due to water mixing within the pond, whose surface was colonized by the yellow waterlily only for one third; this result could be also due to the plant typology, characterized by a low underwater biomass (Horppila & Nurminen 2005; Zbikowski et al., 2010). The dissolved gases in the water column didn't show any difference between levels, except in the case of the methane, where the concentration was higher at the surface of the vegetated site. This could be due to some CH₄ lacunar transport by the submerged leaves (Dacey and Klug, 1979; Heilman and Carlton, 2001) or to some accumulation in superficial water due to leaves cover. We expected to find remarkable differences in oxygen and methane concentrations at the bottom level of the two sites, because of a radial oxygen loss from the rhizome and roots of *N. luteum* and subsequent methane oxidation (Grosse et al., 1996). In fact, it is known the internal atmosphere of the aerenchyma is constituted by atmospheric oxygen, which undergoes to fluctuations along the day and according to the gas flow direction (Lain 1940; Dacey & Klug, 1979). The methane concentrations at the bottom of the vegetated site were instead only slightly diminishing along the day, and values on the whole were not significantly different than in waters overlying bare sediment in the unvegetated portion of the pond. Those results, coupled to extremely low oxygen values, indicated hypoxic conditions even in the presence of the rhizome of the plant. Some authors (Laing 1940a, 1941; Smits et al., 1995) already reported that *N. luteum* rhizome is able to respire both aerobically and anaerobically, that only the roots strictly need oxygen for their survival and that the shoot germination can take place in absence of oxygen. Our result also agree with those reported by Bedford et al. (1991) on other wetland plants, mainly helophytes, whose presence had the net effect of not oxygenating the sediment nor the water. The authors suggested the most of the oxygen consumption was occurring within the root system as respiration. Other than that, our hypothesis is that the roots of *N. luteum* could present a 'tight' barrier to ROL (*sensu* Colmer, 2003), so that longitudinal O₂ diffusion in the aerenchyma is enhanced towards the apex, by diminishing leakage to the rhizosphere. Seago et al. (2000) already reported that *Nymphaea odorata* roots present a thick exodermis, structure that could lead to a high barrier to ROL.

8. Influence of different hydrophyte communities on dissolved gas concentrations and potential trace gas emission from wetlands

8.1 Aim of the study

Oxygen depletion in water column is a common trait of shallow freshwaters colonized by pleustophytes (Morris & Barker, 1977; Pokorný & Rejmánková, 1983; Caraco & Cole, 2002; Parr & Mason, 2004; Bolpagni et al., 2007), whereas other hydrophytes are recognized to have both positive and negative effects on water and sediment oxygenation (Bedford et al., 1991; Colmer, 2003). Even though oxygen depletion is by now an evidence in environments colonized by pleustophytes, little has been done to investigate the effect of anoxia determined by those plants on greenhouse gas emissions (Bolpagni et al., 2007; Pierobon et al., 2010).

The aim of this study was to investigate whether substantial differences establish between environments colonized by different hydrophytes in terms of water oxygenation, with special concern to the GHG production and efflux towards the atmosphere. Secondly, I wanted to verify if the effect of water and sediment oxygenation due to primary production in water and to ROL are only limited in time (i.e. vegetative period) or do have more prolonged effects over a long term scale (i.e. over an entire year). For this purpose, a set of wetlands has been monitored in 2008-2009 with regards to dissolved gases (O₂, CO₂ and CH₄) in water and some other additional variables. The main hypothesis is that the establishing of monospecific mats of free-floating plants induces anoxia, supersaturates the water with CO₂ and decouples methane production and methane oxidation, resulting in potentially high efflux of GHG to the atmosphere.

8.2 Sampling program and calculations

8.2.1 Water sampling

Sampling activities were conducted on 21 shallow wetlands within the Po River Plain from May 2008 to December 2009 (see study area description in chapter 3). Once a month, a water sample was collected at each site at a depth <0.1 m from at least 1 m from the shore (Figure 8.1). Temperature and pH were immediately recorded, and the sample was treated for further

analyses of dissolved gases (O₂, CO₂, CH₄) and DIC (for detailed analyses description see chapter 4).



Figure 8.1. Winter (on the left) and summer (on the right) sampling of superficial water from shallow wetlands within the Po River Plain.

8.2.2 Primary producers characterization

The sites were divided in four categories, according to the dominance of the primary producer: submerged macrophytes (SM, 3 sites), phytoplankton (PH, 5 sites), floating-leaved macrophytes (FL, 9 sites) and pleustophytes (PL, 5 sites). Those categories were defined prior to the start of the study and were considered suitable also during the winter, when the plants were not present. Floating leaves covering (expressed as % of the surface of the basin) of the water surface was estimated by photographs taken in replicates and revised at the computer. Chlorophyll-*a* (Chl-*a*) concentration was measured in water following the method described in chapter 4.

8.2.3 Gas saturation and theoretical flux calculation

Dissolved gases concentration was normalized for water temperature, and thus solubility, and expressed as saturation, according to the formula 2) reported in chapter 4. Theoretical fluxes from the water to the atmosphere were calculated from the dissolved trace gases (CO₂ and CH₄) concentration measured in every sample. We considered the fluxes being regulated from the Fick's first law for one dimension (Fick, 1855), according to the following formula:

$$3) \quad J_{g;T} = \Delta[g] \times \frac{D_{g;T}}{z}$$

where $J_{g,T}$ is the flux from the water to the atmosphere ($\text{mmol m}^{-2}\text{h}^{-1}$) for a given gas (g) at a given temperature (T); $\Delta[g]$ is the difference between the concentration measured in the sample $[g]_s$ and the theoretical gas concentration $[g]_t$ (see calculation in chapter 4); $D_{g,T}$ is the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) for the considered gas at a given temperature and z is the boundary layer (μm), that is the path the gas molecule should cross to diffuse from the water to the atmosphere. In this study I considered for z a value of $750 \mu\text{m}$, which is generally reported in literature for stagnant waters, in absence of wind (Portielje & Lijklema, 1995). In order to obtain a general equation for each gas diffusion coefficient D , values reported in Winkelmann (2007) were plotted *vs.* the water temperature and the following equations were obtained:

$$D_{\text{CO}_2} = 5 \times 10^{-7}(T) + 7 \times 10^{-6} \quad (R^2=0.85, p<0.001, n=53)$$

$$D_{\text{CH}_4} = 3 \times 10^{-6}(T) + 9 \times 10^{-5} \quad (R^2=0.94, p<0.001, n=21)$$

where D is expressed in $\text{cm}^2 \text{s}^{-1}$ and T is expressed in $^\circ\text{C}$.

Positive gas fluxes are to be intended as directed from the water surface to the atmosphere and *vice versa*. Daily values ($\text{mmol m}^{-2}\text{d}^{-1}$) were obtained by converting and averaging hourly fluxes measured during three macroperiods, which were chosen arbitrarily *a posteriori* according to the vegetative stage of the primary producers, as follows:

- Early summer, from April to June (floating leaves cover $<60\%$) = 90 days
- Late summer, from July to September-October (floating leaves cover $>60\%$) = 90-120 days
- Winter, from October-November to March (no plants) = 150-180 days

Differences in late-summer and winter periods are due to a different plants covering at the pleustophytes dominated sites (PL sites), where until the month of October, the plants covered more than 80% of the water surface.

Hourly flux values could be extended to daily values, given that all samplings were performed during the daylight, and thus assuming that values could not be overestimated. Moreover, as the ebullition rates were not quantified in this study, the fluxes are likely to be strongly underestimated (Huttunen et al., 2003). Theoretical fluxes were calculated also when samplings were performed under the ice and floating leaves cover, assuming the degassing to

the atmosphere to happen at the ice melt moment or during the collapse of the vegetation (Michmerhuizen et al., 1996; Huttunen et al., 2003; Song et al., 2008).

Daily values were then summed in order to obtain an annual fluxes estimation ($\text{mmol m}^{-2}\text{y}^{-1}$); in order to evaluate the GHG emission towards the atmosphere, annual fluxes were converted into grams of carbon dioxide equivalents ($\text{g CO}_2\text{-eq m}^{-2}\text{y}^{-1}$) accordingly to the GWP (Global Warming Potential) values reported by IPCC (2001) of 1 and 25, for CO_2 and CH_4 respectively. The GHG emission budget was then assessed for all primary producer categories by summing the contribution of the two greenhouse gases in terms of grams of CO_2 equivalents.

8.3 Results

8.3.1 Primary producers characterization

The covering of the water surface by floating leaves changed along the year with a peak of $80.0\pm 10.0\%$ in June 2009 at the FL sites and a maximum of $100.0\pm 0.0\%$ in September and October of both years at the PL sites. The SM and PH sites didn't present any leaves cover. An ice cap occurred during the winter and lasted for about 50 days at all sites during January-February (Figure 8.2). Chl-*a* concentration varied with a seasonal pattern at the PH, FL, and PL sites: maximum values among the three categories, however, showed at different times during the year. Mean values were comprised between 3.8 ± 1.5 and $162.7\pm 64.4 \mu\text{g L}^{-1}$, measured at the PH sites in September 2008 and August 2009, respectively; when compared to the other categories, SM sites were characterized by a lower Chl-*a* concentration, which slightly peaked in summer (Figure 8.2).

8.3.2 Water chemistry

Dissolved inorganic carbon (DIC) concentrations, pH values and water temperatures ($^{\circ}\text{C}$) are reported in figure 8.3. Water temperature followed a unimodal pattern, with a summer peak, and values comprised between 1 and 35 $^{\circ}\text{C}$. At SM and PH sites, minimum values of DIC were recorded during the summer, corresponding to an increase in pH values. No particular patterns were evidenced at FL sites, for both DIC and pH. At PL sites, DIC pattern was characterized by positive peaks during early autumn, which matched to a decrease in pH, with lower values than those recorded in the sites of the other three categories (rmANOVA, $p<0.01$; TukeyHSD, $p<0.01$).

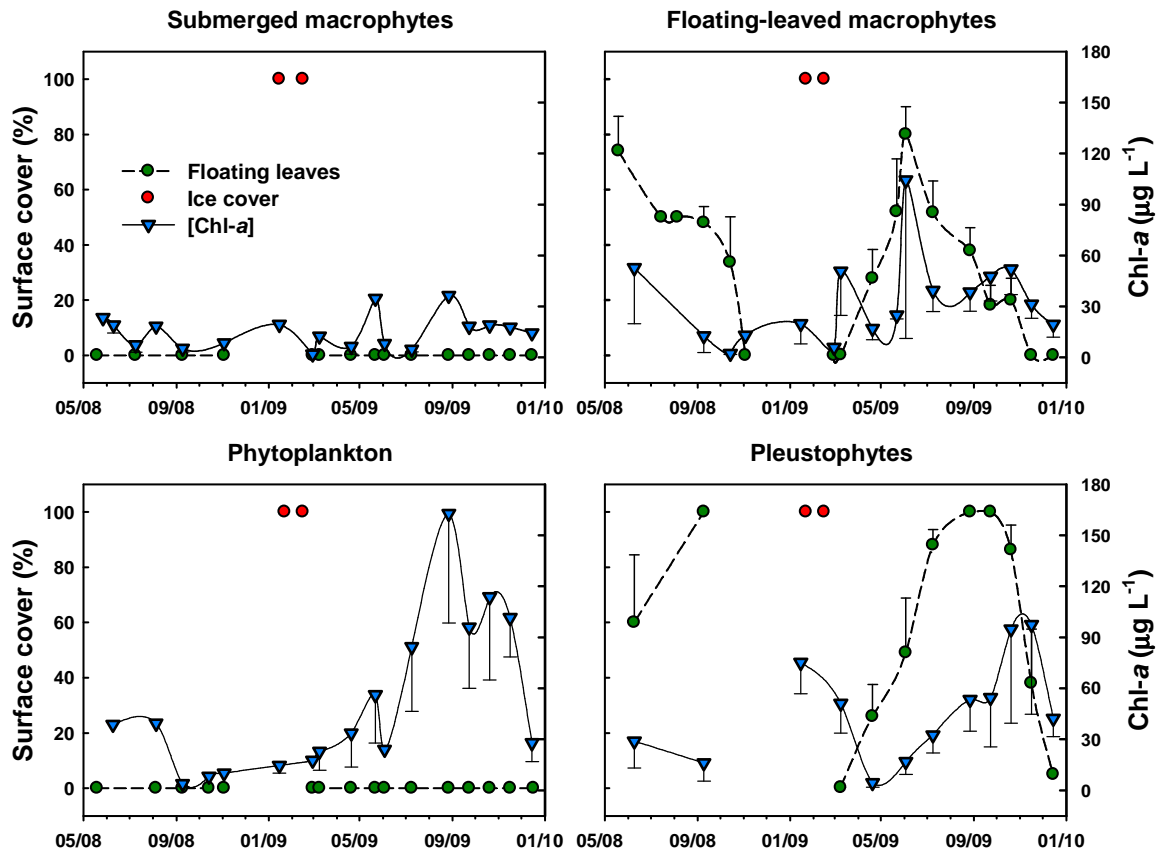


Figure 8.2. Surface cover (%; left axis) and chlorophyll-*a* concentration ($\mu\text{g L}^{-1}$; right axis) measured along the 2008-2009 at 21 shallow freshwater wetlands. Data are divided according to the dominant primary producer (submerged macrophytes, phytoplankton, floating-leaved macrophytes and pleustophytes). The green dots indicate the floating leaves cover, the red dots indicate the ice cover, the blue triangles indicate the Chl-*a* concentration (mean \pm SE; n=3-9).

8.3.3 Dissolved gases saturation in water

Along the duration of the study, most wetlands exhibited O_2 deficiency (66% of sites with values under 100% saturation) at all seasons, with values comprised between 0.01 and 0.74 mM. The lowest and the maximum values were recorded during the winter and summer, respectively, at all sites, except at the PL ones, where the minimum was measured during late summer and autumn (Figure 8.4). Generally, the PL sites significantly differentiated from the other three categories, which presented similar values among them (rmANOVA, $p < 0.01$; TukeyHSD, $p < 0.01$) (Figure 8.5).

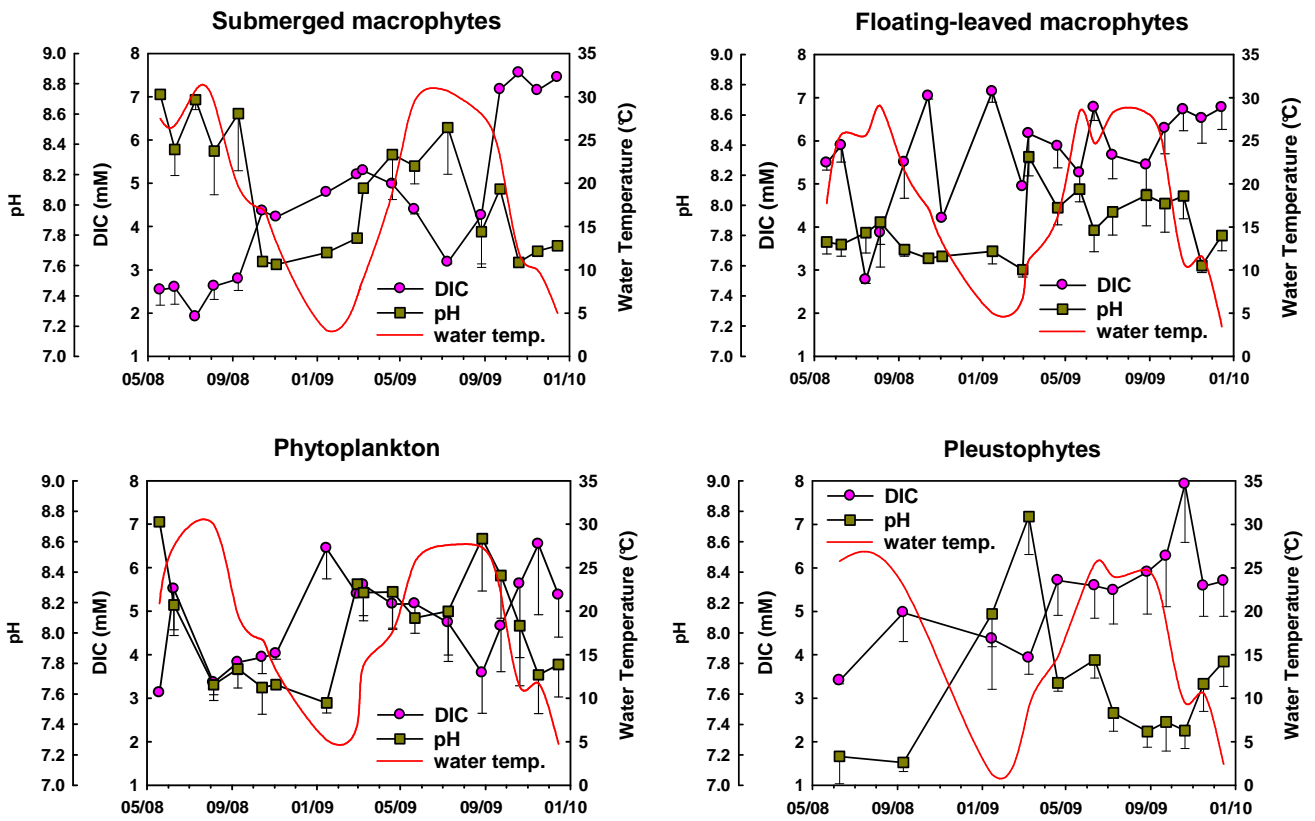


Figure 8.3. Dissolved inorganic carbon (DIC) concentration, pH values and water temperature (°C) measured in 2008-2009 at 21 shallow freshwater wetlands dominated by different primary producers (submerged macrophytes, phytoplankton, floating-leaved macrophytes and pleustophytes) (mean±SE; n=3-9).

All the sites resulted supersaturated in CO₂ and CH₄ at all seasons, with the PL sites forming a separated group when compared to the other three categories of primary producers (rmANOVA, p<0.01; TukeyHSD, p<0.01) (Figure 8.5). Carbon dioxide concentration were comprised between 0.01 and 0.76 mM, and peaked during the winter at SM and PH sites. FL sites didn't present any clear pattern along the year; PL sites distinctly showed a peak during the end of the summer (Figure 8.4). Methane concentration ranged between 0.18 and 173.84 μM; maximum values were recorded at all sites during late summer and autumn, with the PL sites showing much higher values when compared to the other categories (rmANOVA, p<0.01; TukeyHSD, p<0.01) (Figure 8.4). The CO₂ and CH₄ concentrations resulted highly correlated (Pearson's r=0.43, p<0.001); moreover, the two gases resulted inversely correlated to oxygen concentration (Pearson's r=-0.53 and r=-.28, for CO₂ and CH₄, respectively, p<0.001 for both).

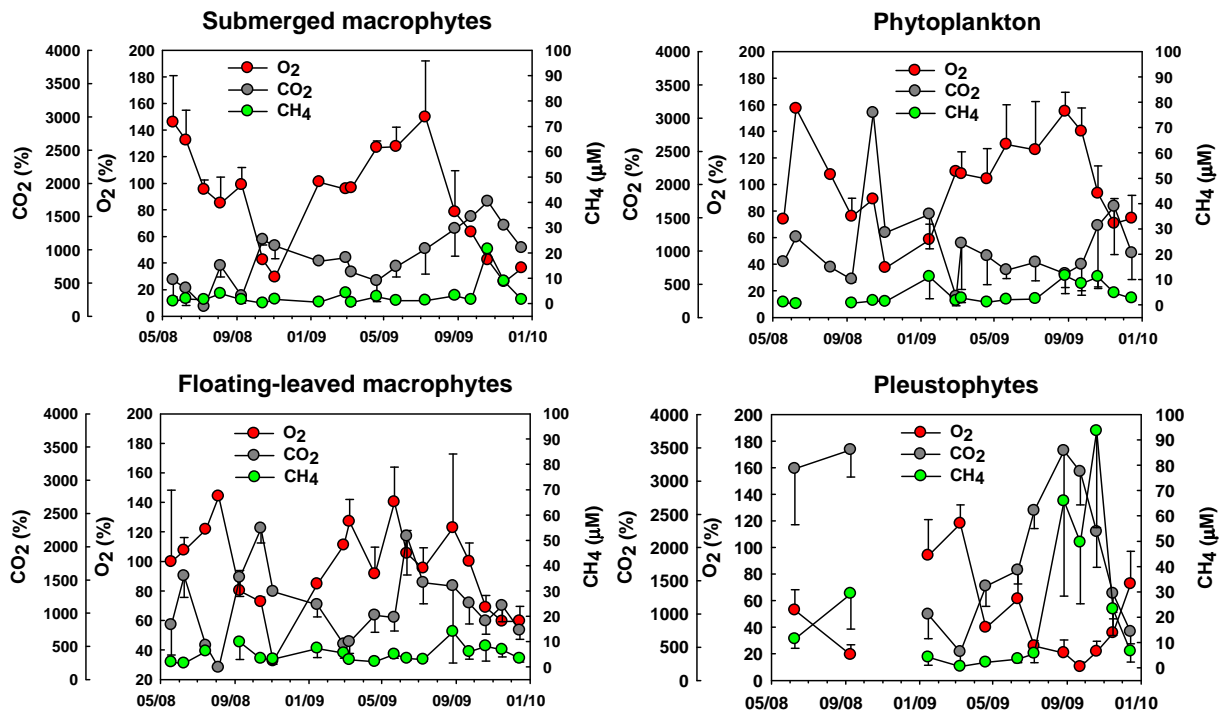


Figure 8.4. Dissolved oxygen, carbon dioxide (expressed as saturation, %, left axes) and methane (expressed as concentration, μM , right axis) patterns measured in 2008-2009 at 21 shallow freshwater wetlands dominated by different primary producers (submerged macrophytes, phytoplankton, floating-leaved macrophytes and pleustophytes) (mean \pm SE; n=3-9).

8.3.4 Diffusive fluxes towards the atmosphere

Being the sites supersaturated both in CO_2 and CH_4 , the calculated gas fluxes resulted constantly positive, that indicating an efflux of greenhouse gases from the water towards the atmosphere. The flux patterns are not reported in graphs because they mostly depend on the changes of the gas concentration in water, as the influence of water temperature is small due to little differences between sites. Hourly mean CO_2 and CH_4 fluxes, calculated over each month, are reported in Table 5.1; the overall values were comprised between 0.08 ± 0.02 and 4.74 ± 1.62 $\text{mmol CO}_2 \text{ m}^{-2}\text{h}^{-1}$ and between 0.02 ± 0.02 and 5.41 ± 2.26 $\text{mmol CH}_4 \text{ m}^{-2}\text{h}^{-1}$. For both gases, minimum fluxes were calculated during the summer at SM sites, while the maximum values were calculated during the autumn at PL sites. Daily values according to different periods of the vegetative stage of the primary producers are reported in Table 8.2. When extended to annual values, the sites resulted emitting between 307.8 ± 49.5 and 712.4 ± 105.4 $\text{mmol CO}_2 \text{ m}^{-2}\text{y}^{-1}$, and between 16.3 ± 4.2 and 183.2 ± 52.4 $\text{mmol CH}_4 \text{ m}^{-2}\text{y}^{-1}$. The

greenhouse gas emission budget was of 177.2 ± 27.4 , 285.1 ± 58.3 , 321.9 ± 47.4 and 1243.3 ± 301.8 g CO₂-eq m⁻²y⁻¹, for SM, PH, FL and PH sites, respectively (Figure 8.6).

Table 8.1. Hourly gas (CO₂ and CH₄) fluxes calculated from dissolved gas concentration and water temperature along the 2008-2009. Values are reported according to the primary producer dominance (submerged macrophytes, SM; phytoplankton, PH; floating-leaved macrophytes, FL; pleustophytes, PL); each mean value is obtained from flux calculated on sites sampled on the same month of the year (mean±S.E.; n=3-9).

Sites	Flux range (mmol m ⁻² h ⁻¹)	
	CO ₂	CH ₄
SM (<i>Ceratophyllum demersum</i>)	0.08±0.02 - 1.91±0.01	0.02±0.02 - 0.51±0.01
PH (Phytoplankton)	0.23±0.01 - 1.63±0.42	0.07±1.00 - 0.66±0.28
FL (<i>Nuphar luteum</i> , <i>Nymphaea alba</i>)	0.62±0.18 - 2.66±0.32	0.11±0.02 - 0.93±0.39
PL (<i>Lemna gibba</i> , <i>Lemna minor</i> , <i>Wolffia arrhiza</i> , <i>Spirodela polyrhiza</i> , <i>Salvinia natans</i>)	0.07±0.07 - 4.74±1.62	0.03±0.01 - 5.41±2.26

Table 8.2. Average daily CO₂ and CH₄ fluxes grouped according to three macroperiods of the vegetative stage of primary producers. Values are reported according to the primary producer category (submerged macrophytes, SM; phytoplankton, PH; floating-leaved macrophytes, FL; pleustophytes, PL) (mean±S.E.; n=3-9).

Period	Daily flux (mmol m ⁻² d ⁻¹) according to the plant category				
	SM	PH	FL	PL	
CO ₂	April - June	16.6±3.7	21.3±3.8	33.5±4.0	54.4±12.2
	July - September/October	20.6±3.7	24.9±4.6	33.4±3.8	81.2±7.2
	October/November - March	30.4±3.8	33.3±5.9	25.4±2.8	17.2±4.8
CH ₄	April - June	2.4±0.6	5.2±2.1	4.8±0.7	8.3±2.4
	July - September/October	3.5±0.6	8.4±2.5	12.6±2.8	78.5±21.2
	October/November - March	4.1±1.2	6.8±1.6	6.3±1.5	14.2±5.7

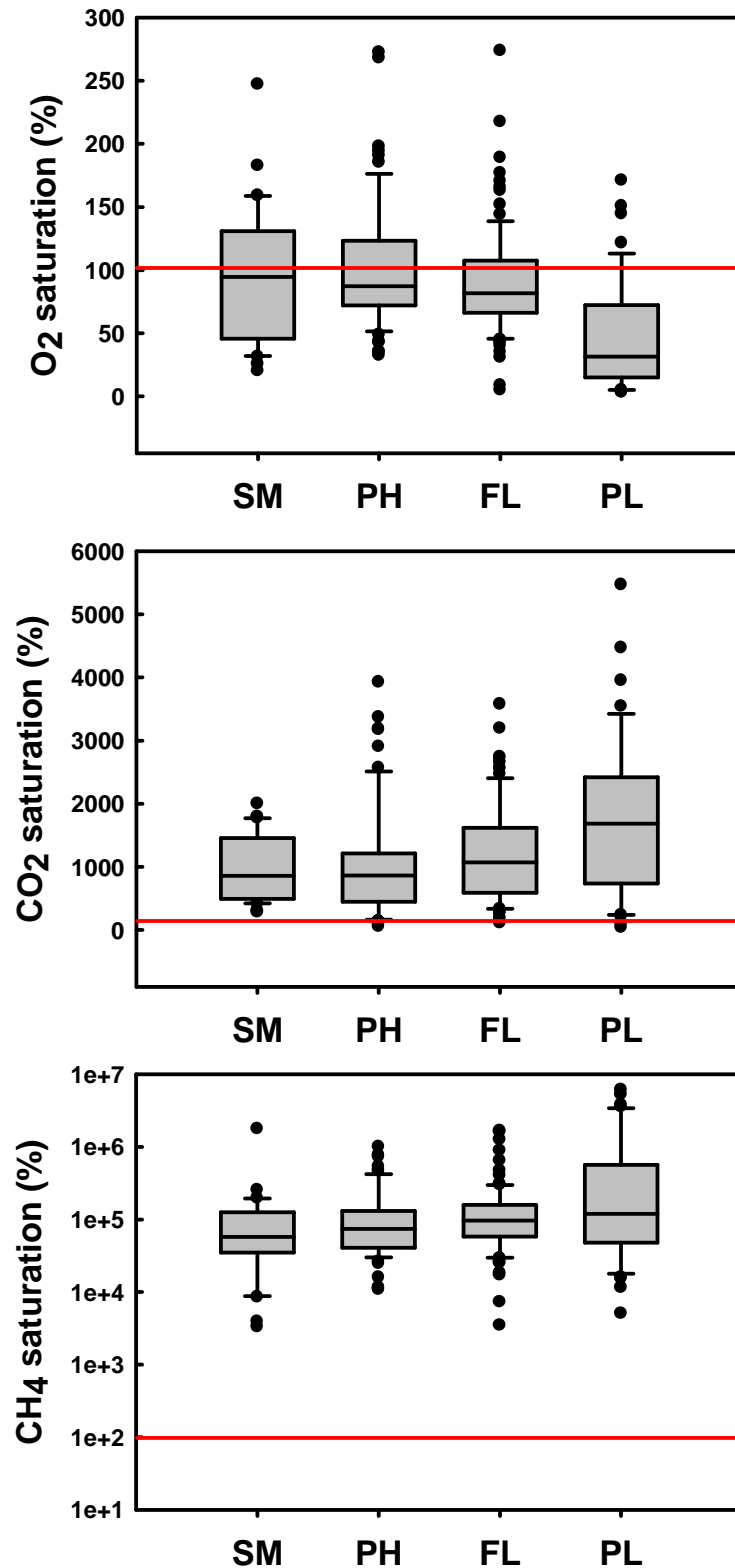


Figure 8.5. Dissolved gases saturation (O₂, CO₂, and CH₄; %) represented according to the dominant primary producer: submerged macrophytes (SM, n=30), phytoplankton (PH, n=68), floating-leaved macrophytes (FL, n=97) and pleustophytes (PL, n=43). The red line indicates the 100% saturation.

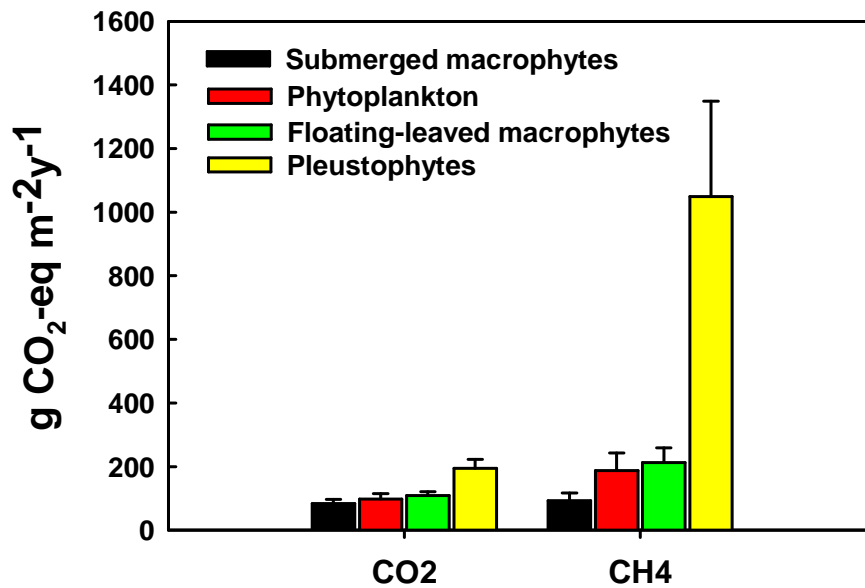


Figure 8.6. Contribution of two greenhouse gases (CO₂ and CH₄) to the radiative forcing of the atmosphere in shallow freshwater wetlands. Values are reported according to the primary producers dominance (mean±S.E.; n=3-9).

8.4 Discussion of the results

In this study the presence of different primary producers was investigated with regards to dissolved gases in water and to the theoretical fluxes of GHG towards the atmosphere. The investigated wetlands were selected since they were characterized by high degree of organic matter in the sediment and nutrient levels in water column, so that to be representative of eutrophic sites.

The results of the study confirm findings from previous investigations on the effects of the floating leaves cover on water oxygenation: the presence of the *Lemnaceae* and aquatic ferns have strong responsibilities on water column anoxia (Pokorný & Rejmánková, 1983; Caraco & Cole, 2002; Parr & Mason, 2004; Bolpagni et al., 2007). The prolonged permanence of the cover on the water surface hinders the oxygen diffusion from the atmosphere and enhance the anaerobic processes. The dissolved CO₂ and CH₄ concentrations measured at PL sites are of order of magnitude higher than those reported in literature for freshwater wetlands: in particular, in the case of methane, I measured concentration that are usually reported for sediment porewater. The methane that accumulates within the water column cannot be

oxidized and is likely released at the moment of the biomass collapse. The leaves cover, excludes the photosynthesis by other primary producers in the water column: from early summer, in fact, the water surface is shaded and obstruct the light penetration. Moreover, the pleustophytes late-summer blooming is the main mechanism with whom they outcompete with the growth of submersed macrophytes and phytoplankton, which typically develop the maximum standing crop between June and August and then decline. From the data concerning the chlorophyll-*a* concentration at PL sites, it is clear that the phytoplanktonic growth is inhibited until the end of September, when, however, the water temperature start to be low for to reach high growth rates; this is in agreement with findings from O'Farrell et al. (2009). In this way, phytoplankton cannot uptake the nutrients in water, which remain available for the growth of pleustophytes. At the same time, the establishing of anoxic conditions along several months during the summer favour the increase of reduction processes, which typically brings to a high mobilization of nutrients from the sediment, which will be available in water also during the successive year (Boedeltje et al., 2004, 2005). This is a positive feedback mechanisms which allows pleustophytes to settle in an eutrophic environment, which will be destined to never come back to a submerged macrophytes-dominance. On the other hand, the presence of submerged macrophytes at some sites, mainly *Ceratophyllum* and *Myriophyllum* spp., didn't bring to a differentiation from the phytoplankton or from the floating-leaved macrophytes dominated sites, in terms of water oxygenation and greenhouse gas production. This is likely due to high sedimentary oxygen demand due to high organic matter within the sediment of those sites; in the same way, respiration processes are so strong that cannot be faced by potential oxidation processes in water column.

Carbon dioxide diffusive fluxes I calculated are comparable to those reported by Casper et al. (2000), while in the case of the methane, values are above the mean values reported for wetlands of the boreal hemisphere, for every category of primary producers (Figure 87). This result could be imputable to the high temperature reached at our sites during the summer, which generates the great difference with measurements performed in North Europe. Nevertheless, the answer could be in the high degree of the respiration processes at our sites deriving from progressive burial due to organic matter accumulation and high nutrient levels: this is probably the direction in which the studies should be focused in the future, in a context of increasing eutrophication in shallow aquatic environments.

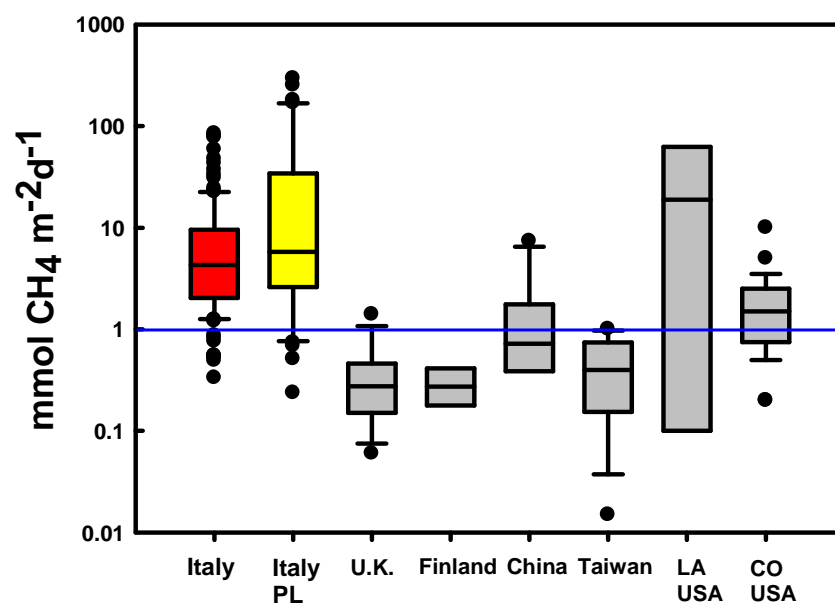


Figure 8.7. Comparison between methane fluxes calculated in this study (red boxplot: SM+PH+FL categories; yellow boxplot: PL category) and values calculated in other studies for shallow freshwater wetlands in the boreal hemisphere. The grey boxplots indicate data taken (from left to right) from Casper et al. (2000), Kankaala et al. (2004), Song et al. (2008), Chang & Yang (2003), Smits & Lewis (1992) and Delaune et al. (1993). The blue line indicates the mean methane emission value reported by Bridgman et al. (2006) for North American freshwater wetlands.

9. Conclusions

The present work focuses on the regulation of GHG emission by different primary producers in shallow wetlands of the Po River Plain. In eutrophic environments the increase of organic matter input favours the shift of primary producer communities. As a consequence of the increase of water turbidity and phytoplankton growth, submerged rooted vegetation is replaced by floating-leaved species. The latter are selected in nutrient-rich and light-limited aquatic bodies.

The main result of the work is that all the investigated sites resulted emitting large amounts of GHG towards the atmosphere, here comprised the two riverine sites. In this work only 21 sites were investigated, but it would be reasonable to hypothesize that most of the stagnant freshwaters of the Po River Plain are in similar conditions, as they are interested by diffuse eutrophication. Both estimated and measured greenhouse gas fluxes indicate higher values than those reported for similar environments of the boreal hemisphere. Those results are likely imputable to the high content of organic matter in the sediment combined to elevated water temperatures, factors which enhance respiration processes. At some sites, those results were promoted by the presence of pleustophytes, which determined the establishing and persistence of hypoxic conditions. Pleustophytes dominated sites resulted being the most concerning situation when compared to other plant communities: the entity of GHG supersaturation and the temporal dynamics at were strictly indeed dependent upon the floating leaves cover. Those results evidence how further investigations are needed, especially at our latitude, as the spread of free-floating plants in calm waters is strictly coupled to high water and air temperature and high organic matter, creating that an extremely favourable environment to the plants proliferation and the methanogenic activity.

Outcomes from the study on the floating-leaved rhizophyte community underline the need of including the estimates of the carbon fixation when assessing the budget of GHG emissions. When normalized, in fact, the methane emissions mediated by the aerenchyma didn't lead to a higher emission when compared to diffusive processes occurring in the open water. During the vegetative stage (May-August), the carbon fixation by the plants counterbalances the methane emission from the aerenchyma: in this period, the presence of those plants don't influence the role of the wetland in acting as a sink/source of GHG. Nevertheless, there is still the need to account for the winter GHG emission, which could be somehow enhanced by the litter accumulation at the vegetated sites. Moreover, deeper investigations are needed to evidence any possible influence of the oxygen transport to the rhizosphere, as my study

apparently didn't show any strong benefit on methane oxidation in water due to a potential oxygen transport.

The experiments on the rooted submerged macrophytes resulted being the more effective in controlling the methane production from organic-rich sediments: the elevated oxygen loss from roots determined in both studies the drastic decline of methane concentrations in porewater and the consequent decrease of methane fluxes towards the water. Moreover, the oxygen transport capacity of the investigated plant (*V. spiralis*) didn't result to be affected by the increase of organic matter in the sediment we tested in the experimental design. There is thus more need to investigate until which degree of organic enrichment this species is capable to maintain such buffer ability, thus reducing methanogenesis and anaerobic processes strength.

On the whole, results from the present study show that, following the shift of primary producers from submerged to floating forms, an increasing amount of greenhouse gases is released to the atmosphere. This outcome should be of global concern when considering the raising of aquatic environments that suffer from eutrophication and that are undergoing to regime shift; those environments are likely to be colonized by free-floating plants and significantly affect the global trace gas budget.

10. References

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