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CO_2 and CH_4 fluxes across a *Nuphar lutea* (L.) Sm. stand

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ABSTRACT

*Floating-leaved rhizophytes can significantly alter net carbon dioxide (CO_2) and methane (CH_4) exchanges with the atmosphere in freshwater shallow environments. In particular, CH_4 efflux can be enhanced by the aerenchyma-mediated mass flow, while CO_2 release from supersaturated waters can be reversed by the plant uptake. Additionally, the floating leaves bed can hamper light penetration and oxygen (O_2) diffusion from the atmosphere, thus altering the dissolved gas dynamics in the water column. In this study, net fluxes of CO_2 and CH_4 were measured seasonally across vegetated [*Nuphar lutea *(L.) Sm.] and free water surfaces in the Busatello wetland (Northern Italy). Concomitantly, dissolved gas concentrations were monitored in the water column and* N. lutea *leaf production was estimated by means of biomass harvesting. During the vegetative period (May-August), the yellow waterlily stand resulted a net sink for atmospheric carbon (from 97.5 to 110.6 g C-CO_2 m^{-2}), while the free water surface was a net carbon source (166.3 g C-CO_2 m^{-2}). Both vegetated and plant-free areas acted as CH_4 sources, with an overall carbon release comprised between 71.6 and 113.3 g C-CH_4 m^{-2}. On the whole, water column chemistry was not affected by the presence of the floating leaves; moreover, no significant differences in CH4 efflux were evidenced between the vegetated and plant-free areas. In general, this study indicates that the colonization of shallow aquatic ecosystems by* N. lutea *might not have the same drastic effect reported for free-floating macrophytes.*

Key words: methane, carbon dioxide, dissolved oxygen, leaf production.

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INTRODUCTION

Eutrophication processes in freshwater wetlands and littoral areas lead to pronounced shifts in primary producer communities (Scheffer et al. 1993, 2001). In turbid and nutrient-rich systems, competition for light among different macrophyte species results in the differentiation of canopy structures and related photosynthetic processes. Floating-leaved rhizophytes and free-floating plants are favored over submerged forms thanks to direct light and atmospheric carbon dioxide uptake (Portielje, and Roijackers 1995; Scheffer et al. 2003; Pierobon et al. 2010). In eutrophic shallow areas, floating-leaved plants can suppress up to 99% of the incident light, thus limiting or excluding phytoplankton and submerged vegetation (Janes et al. 1996; Scheffer et al. 2001; Parr et al. 2002).

The shift from submerged to floating-leaved rhizophytes has relevant consequences for the dissolved gas dynamics. In fact, most of the oxygen (O_2) produced during photosynthesis by floating-leaved plants is released to the atmosphere, while floating leaves hamper underwater photosynthesis as well as atmospheric O_2 diffusion, thus promoting water hypoxia (Pokorný, and Rejmánková 1983; Parr et al. 2002; Bolpagni et al. 2007; Goodwin et al. 2008). At the same time, dissolved organic matter from root exudates and from the decaying litter fuels heterotrophic activity (Caraco, and Cole 2002; Pełechaty 2007; Longhi et al. 2008). Organic matter accumulation under low oxygen conditions results in large methane (CH_4) production within sediment coupled to limited methane reoxidation; as long as the plant canopy represents a barrier against the escape to the atmosphere, the supersaturation of this gas in the water column is expected (Bolpagni et al. 2007). Analogously, carbon dioxide (CO_2) accumulation below the plant canopy is likely to occur, due to limited atmospheric evasion of CO_2 released from sediment (Caraco, and Cole 2002; Williams, and Hecky 2005; Bolpagni et al. 2007). On the other hand, floating-leaved rhizophytes have lacunal tissues (aerenchyma), which act as an air chamber for floating and permit pressurized ventilation between leaves and rhizomes/roots. The aerenchyma-mediated mass flow of oxygen from leaves to rhizosphere is accompanied by methane direct release from sediments to the atmosphere (Ohno 1910; Dacey, and Klug 1979; Schröder et al. 1986; Große, and Mevi-Schutz 1987; Smith, and Lewis 1992; Yavitt, and Knapp 1998). Methane emission rates have also been related to plant production (Schütz et al. 1989; Whiting, and Chanton 1993). Concurrently, pressurized ventilation and radial oxygen loss (ROL) can stimulate CH_4 oxidation, counterbalancing the potential accumulation of this gas in the water and its emission to the atmosphere (Dacey 1980; Dacey, and Klug 1982b; Große et al. 1996; Larmola et al. 2003; Ribaudo et al. 2011).

Monospecific stands of floating-leaved plants have thus a potential impact on water oxygenation and on the accumulation/release of CH_4 and CO_2, two among the most important greenhouse gases (GHG) (Smith, and Lewis 1992; Kankaala et al. 2004; Wang et al. 2006). While a wealth of studies have dealt with CH_4 measurements in helophyte stands (van der Nat, and Middelburg 1998; Brix et al. 2001; Kankaala et al. 2005; Laanbroek 2010), few data are available for floating-leaved rhizophytes (Dacey, and Klug 1979; Smith, and Lewis 1992; Juutinen et al. 2003; Kankaala et al. 2003). Moreover, in a very limited number of studies the magnitude and seasonal trends of CO_2 and CH_4 fluxes across the vegetated surface were measured simultaneously (Bolpagni et al. 2007).

In this study, we aim at evaluating if and how a monospecific stand of a floating-leaved rhizophyte [*Nuphar lutea* (L.) Sm.] affects the concentrations of dissolved O_2, CO_2 and CH_4 in the water column and the net fluxes of CO_2 and CH_4 across the water-atmosphere interface compared to a vegetation-free adjacent area. Previous studies have found that CH_4 efflux from *Nuphar* stands accounted for a large fraction of the total wetland emission (Dacey, and Klug 1979) and that emission from the macrophytes were significantly higher than those from the open water sites (Smith, and Lewis 1992). We hypothesize that the yellow waterlily stands promote hypoxic conditions, CH_4 and CO_2 supersaturation and large methane effluxes, but we also postulate that CO_2 efflux from supersaturated water can be mitigated through fixation by the floating leaves, resulting in net CO_2 uptake. We then hypothesize that differences between vegetated and free water surface follow daily and seasonal patterns, depending on water temperature, vegetation phenology, productivity and metabolism. These issues are relevant at a larger scale due to two main reasons. 1) Eutrophication is expected to enhance the distribution of floating-leaved rhizophytes and the occurrence of hypoxia/anoxia in aquatic habitats, resulting in uncoupled production and oxidation of anaerobic metabolism end-products (Scheffer et al. 2003). 2) The role of freshwater vegetated habitats as sources or sinks of GHG is still poorly explored in the scientific literature, despite the large surfaces occupied by freshwater marshes and small lakes, which account for more than 38,000 and 2,000,000 km^2 in Europe and in North America, respectively (Bridgham et al. 2006; Saarnio et al. 2009; Bastivken et al. 2011).

MATERIALS AND METHODS

Study area and sampling schedule

The Paludi di Ostiglia marsh is a Natural Reserve of 81 ha located between the provinces of Mantua and Verona, Northern Italy (45°6'16"N, 11°5'59"E). It hosts a network of shallow ponds and narrow channels, whose overall mean depth is 0.9 m. Aquatic vegetation is composed by stands of common reed (Phragmites australis (Cav.) Trin. ex Steud.), mats of floating-leaved macrophytes as *Nuphar lutea* (L.) Sm. and *Nymphaea alba* (L.) and residual meadows of *Ceratophyllum demersum* (L.), *Myriophyllum spicatum* (L.) and *Najas marina* (L.). In summer, the water surface is widely colonized by the invasive aquatic fern *Salvinia natans* (L.) All. This marsh is undergoing rapid infilling and exhibits elevated organic matter content in surface sediments (30% as LOI, loss of ignition). High rates of benthic respiration determine night hypoxia during summer months, when water temperature can reach 35°C (Longhi et al. 2008). Nitrate, ammonium and soluble reactive phosphorous average 40, 10 and <1 μM, respectively, on an annual scale (Longhi et al. 2008; Ribaudo, unpublished data).

The study was carried out within a 0.2 ha pond located in the central portion of the marsh; approximately half of the water surface was covered by a monospecific stand of *N. lutea*, while the remaining was devoid of macrophyte vegetation. Sampling campaigns were carried out during the different phases of the life cycle of *N. lutea*: germination (May 2009), biomass peak (July and August 2008, August 2009) and senescence (September 2008) (Tab. 1). Each sampling campaign consisted of a 24-h cycle of measurements performed from a small boat on the vegetated and plant-free water surface. The only exception was July 2008, when measurements were not performed on the free water surface. Two sampling stations (N1 and N2) were chosen within the *N. lutea* stand, while one station (W) was chosen within the area devoid of plants. Each station was equipped with a simple docking formed by three poles, in order to maintain the same boat positioning through-out the campaigns, and to perform water samplings and gas flux measurements approximately in the same area. Water samplings at the vegetated area were performed in between the N1 and N2 stations.

Water sampling and analyses

In the centre of the *N. lutea* stand (station: N1-N2) and of the vegetation free area (station: W) a pipe was inserted into the sediment. The pipe had two water sampling ports, one located approximately 2 cm above the sediment surface (depth: Bottom) and the other 2 cm below the water-atmosphere interface (depth: Surface), so that the sampling depth resulted in 60 and 90 cm at the *N. lutea* and free water stations, respectively. During each sampling campaign, four water samples (two depths and two stations) were collected in three replicates with 100 ml plastic syringes at 3-4 h intervals over 24 hours. Immediately after sampling, an unfiltered aliquot was transferred into a beaker where water temperature and pH were measured with a YSI Multiple Probe (model 556). Another unfiltered aliquot was transferred into 12 mL glass vials (Exetainer Labco, High Wycombe, UK) and Winkler reagents were added for dissolved O_2 determination. Gas samples for dissolved CO_2 and CH_4 analyses were obtained using the headspace technique (McAuliffe 1979) by transferring a 600 mL water sample into a heavy-walled glass 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 bottle head-space by means of plastic syringes (60 mL) and carefully flushed into 12 mL non-evacuated vials (Exetainer Labco, High Wycombe, UK). In addition, surface water samples were collected in three replicates at both sites and filtered with Whatman GF/F filters for chlorophyll a (Chl-*a*) determination. Filters were transferred in polycarbonate vials and kept refrigerated in a cooled box.

Dissolved O_2 was determined by means of iodometric titration (Winkler 1888). CH_4 analyses were performed with a Fisons 9000 gas chromatograph equipped with a flame ionization detector (FID). CO_2 analyses were performed with a Thermo-Finnigan Trace gas chromatograph equipped with a thermal conductivity detector (TCD). Gas partial pressures were converted into concentrations in water using Henry's constant, water temperature, the measured gas partial pressures in air and taking into account the volume of water and headspace inside the bottle (Sander 1999). Gas saturation was calculated as the ratio between the measured gas concentration and that expected if the water sample was in equilibrium with an atmosphere containing 1780 ppb CH_4 and 380 ppm CO_2 (Forster et al. 2007). Chl-*a* concentration was measured spectrophotometrically after extraction with 90% acetone (Lorenzen 1967).

CO_2 and CH_4 flux measurements

Gas flux measurements (CO_2 and CH_4) across the water-atmosphere interface were performed by means of floating static chambers, which were gently laid on the vegetated and free water surface from the boat. One chamber was used to measure fluxes across the vegetation-free water surface (station: W) while two chambers were used for gas flux measurements in the presence of *N. lutea* (stations: N1 and N2). Each chamber was made of transparent plexiglas and was equipped with two 12V fans and with an airbag connected to the exterior by a tube, in order to avoid underpressure in the chamber head-space during gas sampling. Each chamber had an inner volume of 0.128 m^3 and a total area of 6400 cm^2.

On each sampling campaign and station, measurements were repeated every 3-4 hours over a 24 h cycle to cover the diurnal variations of fluxes. Gas samples were collected at 0, 3, 6 and 10 minutes after the chamber positioning. Gas were sampled with a plastic syringe (60 mL) connected to a plastic tube and carefully flushed (~5×) in 12 mL non-evacuated vials (Exetainer Labco, High Wycombe, UK). Samples were kept in a refrigerated box until analyzed in the laboratory, generally within 2-3 days, according to the methods previously reported. Photosynthetically active radiation (PAR) was monitored every 20 minutes all day long with a PAR quanto-photo-radiometer (Delta OHM, HD9021 model); PAR values were recorded every 3 minutes during gas flux measurements.

Water-atmosphere fluxes of CO_2 and CH_4 (mmol m^{-2} h^{-1}) were calculated with a linear regression of concentrations *versus* incubation time; positive fluxes indicate a flux from the free or vegetated water surface to the atmosphere. Fluxes were considered reliable when presenting a R^2 >0.80. The short incubations allowed to quantify only diffusive fluxes or gas exchanges mediated by *N. lutea*, whilst gas evasion via ebullition was not considered. Fluxes in the light period (mmol m^{-2} d^{-1}) were calculated as the sum of the products of hourly fluxes measured during daylight by the time length between two subsequent measurements. Fluxes in the dark phase (mmol m^{-2} d^{-1}) were calculated as the sum of the products of hourly fluxes measured during the night by the time length between two subsequent measurements. Daily fluxes (mmol m^{-2} d^{-1}) were then calculated as the sum of light and dark fluxes. As measurements in the dark were not performed in July 2008, rates were assumed similar to those measured in the dark period in August 2008. Net carbon fixation during the vegetative period of *N. lutea* was tentatively estimated by integrating daily rates for the vegetative period May-August. This calculation was done assuming constant rates for the periods between successive samplings (namely July 2008=31 days, August 2008=31 days, May-June 2009=61 days and July-August 2009=62 days).

N. lutea biomass and primary production

In August 2008, May and August 2009, the aboveground biomass of *N. lutea*, including floating and submerged leaves, was harvested from triplicate 1 m^2 areas distributed randomly across the vegetated stand. Leaves were kept in tanks filled with water until transfer to the laboratory, where they were rinsed and separated into blades and petioles. Leaf density (LD, leaves m^{-2}) and aboveground biomass (g dry weight (g_{DW}) m^{-2}, after drying at 70°C for 72 hours) were measured. Estimates of blade surface area (LSA, cm^2 $blade^{-1}$) were obtained by considering the blade shape as elliptic and measured as

Tab. 1. Number of *N. lutea* floating leaves per square meter (pooled data from measurements at N1 and N2, mean±SD, n=6-9), leaf density (floating+submerged leaves, mean±SD, n=3), aboveground biomass (mean±SD, n=3), and leaf area index (LAI, floating+submerged leaves) measured on a *N. lutea* stand during five campaigns from July 2008 to August 2009. Biomass values marked with the asterisk (*) are underestimates of true values as they were obtained from the number of floating leaves and do not include those submerged.

Campaign	Floating leaves leaves m^{-2}	Leaf density leaves m^{-2}	Biomass g_{DW} m^{-2}	LAI m^2 m^{-2}
07/16/2008	25.4±1.7	-	176.4±12.1*	0.91±0.14*
08/06/2008	27.3±1.9	29.3±1.9	203.4±12.9	1.05±0.17
09/25/2008	10.3±0.8	-	71.2±5.8*	0.37±0.06*
05/23/2009	11.6±1.6	14.5±1.5	100.9±10.5	0.52±0.07
08/01/2009	30.5±1.3	33.3±3.3	230.5±22.7	1.19±0.19

(length×width)/2; leaf dry weight (mg $leaf^{-1}$) was obtained from five leaves from each replicate. The Leaf Area Index (LAI, m^2 m^{-2}) was calculated by multiplying the average blade surface area by the average number of leaves (floating and submerged) in a square meter. Leaf density and LAI for the campaigns of July and September 2008 were calculated from the number of the floating leaves inside the chamber, which was recorded in correspondence of every gas flux measurement. Petioles were measured from the basis to the blade. Ten replicates of 5 cm long petiole segments were dried and weighed; the mean weight of one leaf (blade+petiole, g_{DW} $leaf^{-1}$) was determined by the sum of the average blade and petiole weight. Biomass values for July and September 2008 were calculated from the number of floating leaves inside the chamber multiplied by the mean weight of one leaf.

Net carbon fixation by the aboveground portion of *N. lutea* was estimated as the leaf production. Daily leaf production rate (LP, leaves m^{-2} d^{-1}) was calculated according to the following formula:

$$LP=LA\times LD \qquad (1$$

where LA=0.05 leaf $leaf^{-1}$ d^{-1} is the leaf growth rate, assuming a production of 1.5 new leaves per rosette per week and an average number of 4 leaves per rosette (Kouki 1991; Titus, and Sullivan 2001); LD is the leaf density (leaves m^{-2}) measured *in situ* considering only leaves with floating blades. This calculation underestimates the true net C fixation as the quota fixed by submerged leaves or translocated to belowground tissues is not taken into account. LP was then multiplied by the mean leaf dry weight (g_{DW} $leaf^{-1}$) and by its mean carbon content (0.38 g C g_{DW}^{-1}; Longhi et al. 2008) in order to calculate the carbon fixed daily by the above-ground portion of *N. lutea* in a square meter (g C m^{-2} d^{-1}). This calculation was not performed with data from September 2008 as the leaves were visibly in a decay phase. Rates of carbon fixation calculated from biomass harvesting were then integrated over the vegetative period as described above and compared with those calculated via gas flux measurements.

Statistical analyses

The influence of the three independent factors sampling station (N1, N2 and W), sampling depth (surface and bottom) and sampling season (spring, summer and autumn) and their interactions were tested on water temperature, pH and concentration of dissolved O_2, CO_2, and CH_4 by means of three-way ANOVA. The effects of sampling station and sampling season and their interaction were tested on Chl-*a* concentration with two-way ANOVA. The effects of the three factors sampling station, irradiance and water temperature were tested on CO_2 and CH_4 fluxes with three-way ANOVA and Tukey's HSD multiple comparison test, with each individual measurement as replicates. The dependence of the gas fluxes on the concentrations in the surface water layer and on *N. lutea* biomass were tested with a linear regression model.

Prior to the analyses, dependent variables were tested for ANOVA assumptions with Shapiro-Wilk and Levene's tests; only CH_4 fluxes needed to be log-transformed for normality requirements. Analyses were performed using the R Program (R - Development Core Team 2011); the α value was set to 0.05.

RESULTS

N. lutea biomass and primary production

The vegetative cycle of the yellow waterlily lasted about 4 months and was characterized by leaf emergence in early May, a peak biomass in July-August and decay phase starting in September (Tab. 1). The estimated mean blade surface area was 357±25 cm^2, the average petiole length was 93.4±5.5 cm and the mean dry weight of a leaf was 6.93±0.43 g_{DW} (pooled data from all samplings); leaf area index varied between 0.37±0.03 and 1.19±0.08 m^2 m^{-2} (Tab. 1). During spring and summer measurements,

Tab. 2. Calculated daily leaf production (mean±SD, n=3) and net carbon fixation by the yellow waterlily. Daily leaf production was calculated from measured leaf density (pooled data from stations N1 and N2) and a leaf growth rate of 0.05 leaf leaf^{-1} d^{-1} (Kouki 1991; Titus & Sullivan 2001).

Campaign	Daily leaf production (LP) leaves m^{-2} d^{-1}	Net carbon fixation from leaf production g C m^{-2} d^{-1}	from CO$_2$ fluxes g C m^{-2} d^{-1}
07/16/2008	1.3±0.0	3.4±0.1	0.6±0.4
08/06/2008	1.4±0.0	3.6±0.1	0.8±0.1
09/25/2008	0.5±0.0	-	-0.9±0.9
05/23/2009	0.6±0.0	1.5±0.1	1.2±0.5
08/01/2009	1.6±0.1	4.1±0.2	0.5±0.3

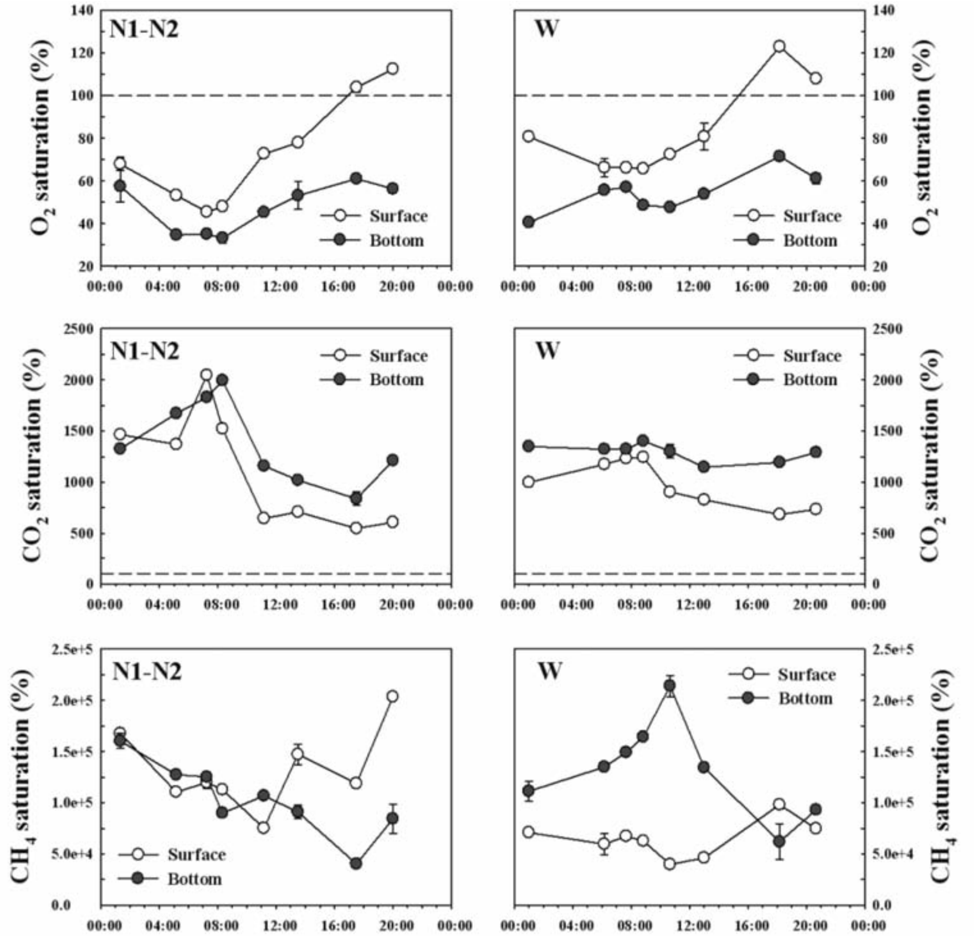

Fig. 1. O$_2$, CO$_2$ and CH$_4$ saturation values (mean±SD, n=3) measured in surface and bottom water samples from a *N. lutea* vegetated stand (N1-N2) and an adjacent area devoid of plants (W). The dashed line refers to 100% saturation. Data from the August 2009 sampling campaign are reported, as representative of the daily dissolved gas dynamics.

N. lutea blades looked green and healthy, whereas in September the stand consisted mostly of old blades, visibly damaged by the gnawing of the waterlily beetle *Galerucella nymphaeae*. We calculated for the vegetative phase of *N. lutea* a production of 81.8±0.1 leaves m^{-2} in the period July-August 2008 (62 days) and a production of 132.1±0.2 leaves m^{-2} in the period May-August 2009 (123 days) (Tab. 2). The net carbon fixation estimated from leaf production varied between 1.5±0.1 and 4.1±0.2 g C m^{-2} d^{-1}, calculated for May and August 2009, respectively (Tab. 2). Carbon fixed by the aboveground portion of *N. lutea* was thus estimated in 215.6±3.8 g C m^{-2} for the period July-August 2008 and 348.1±10.5 g C m^{-2} for the period May-August 2009.

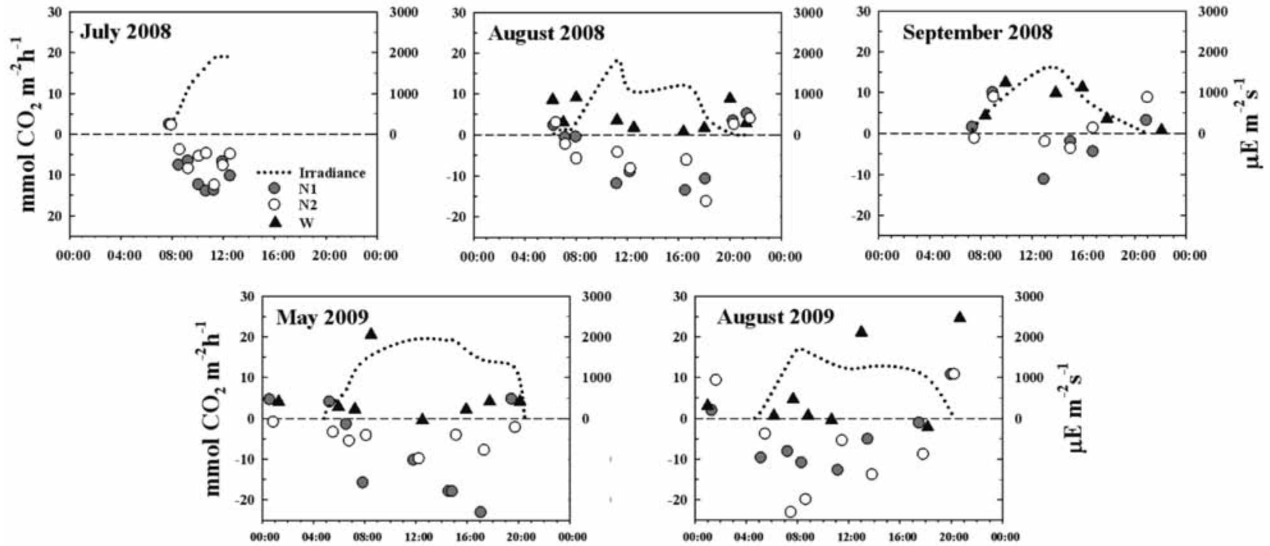

Fig. 2. Hourly CO_2 fluxes measured on a *N. lutea* stand (N1 and N2 stations) and on the free water surface (W station) during seasonal 24 h cycles. Note that the measurements performed in July 2008 refer only to daylight. Irradiance (PAR) is reported on the dotted line and refers to measurements recorded every 20 minutes all day long and every 3 minutes during measurements.

Water features

At the sampling site, water temperature varied seasonally, was homogeneous along the vertical profile and was not significantly different in the presence or absence of *N. lutea*. Average daily values varied between a minimum of 17.0±0.3 and a maximum of 29.9±0.5°C measured in September and August 2008, respectively, with summer peaks up to 34°C measured at midday. pH was rather constant throughout the sampling period with higher values in the surface (8.0±0.1 at both stations) compared to the bottom water layers (7.6±0.1 at both stations). Differences between vegetated and unvegetated areas were not significant. The Chl-*a* concentration varied significantly with sampling periods but it was not statistically different between sampling stations. The highest values were measured in August 2009 (26.4±1.3 $\mu g\ L^{-1}$, pooled data), while lowest values were measured in September 2008 (2.4±2.0 $\mu g\ L^{-1}$, pooled data).

An example of gas (O_2, CO_2 and CH_4) saturation daily pattern is reported for August 2009, at peak water temperature and *N. lutea* biomass (Fig. 1). The concentrations of dissolved O_2 and CO_2 were significantly affected by the factor "water depth" but they were not statistically different in the presence or absence of *N. lutea*. Dissolved O_2 exhibited relatively low concentrations in water layers adjacent to sediments, with an average value for the sampling period of 0.16±0.01 mM (pooled data from the two sampling stations), corresponding to an average saturation of 64±5%. In surface water layers dissolved oxygen concentrations were significantly higher, with an average value of 0.22±0.01 mM, corresponding to a saturation of 90±4%. Dissolved CO_2 concentrations followed an opposite pattern with higher values on the bottom of the water column (0.15±0.01 mM) compared to the surface (0.11±0.01 mM). Dissolved CO_2 concentration was always much higher than theoretical equilibrium values; its saturation was inversely correlated with that of O_2 (R^2=0.47, p<0.001, n=131). Dissolved CH_4 was also much above saturation at both vegetated and unvegetated stations and did not show any clear seasonal trend, nor any systematic difference between bottom (1.88±0.14 µM, pooled data) and surface water layers (2.12±0.27 µM, pooled data). There was a tendency towards higher CH_4 concentration in the water of the vegetated station (2.39±0.26 µM, pool of surface and bottom data) compared to the vegetation-free area (1.54±0.16 µM, pool of surface and bottom data) but, as for dissolved oxygen and carbon dioxide, differences were not statistically significant.

Gas flux measurements

CO_2 fluxes were significantly different at the vegetated and free water stations but differences depended upon the interaction term "station×irradiance" (Tab. 3). Hourly CO_2 fluxes measured at station W were predominantly directed towards the atmosphere and ranged between 2.20 and 24.57 mmol $CO_2\ m^{-2}\ h^{-1}$ (Fig. 2). Here, carbon dioxide fluxes were not significantly correlated with the concentration of the gas in the surface water, nor with irradiance or water temperature. On a daily basis, the vegetation-free water surface was a net CO_2 source with slight differences between sampling periods; rates varied between 108.6 and 119.4 mmol $CO_2\ m^{-2}\ d^{-1}$ (Fig. 4).

In the *N. lutea* stand, net CO_2 uptake was measured in the light for both N1 and N2, with September 2008 as

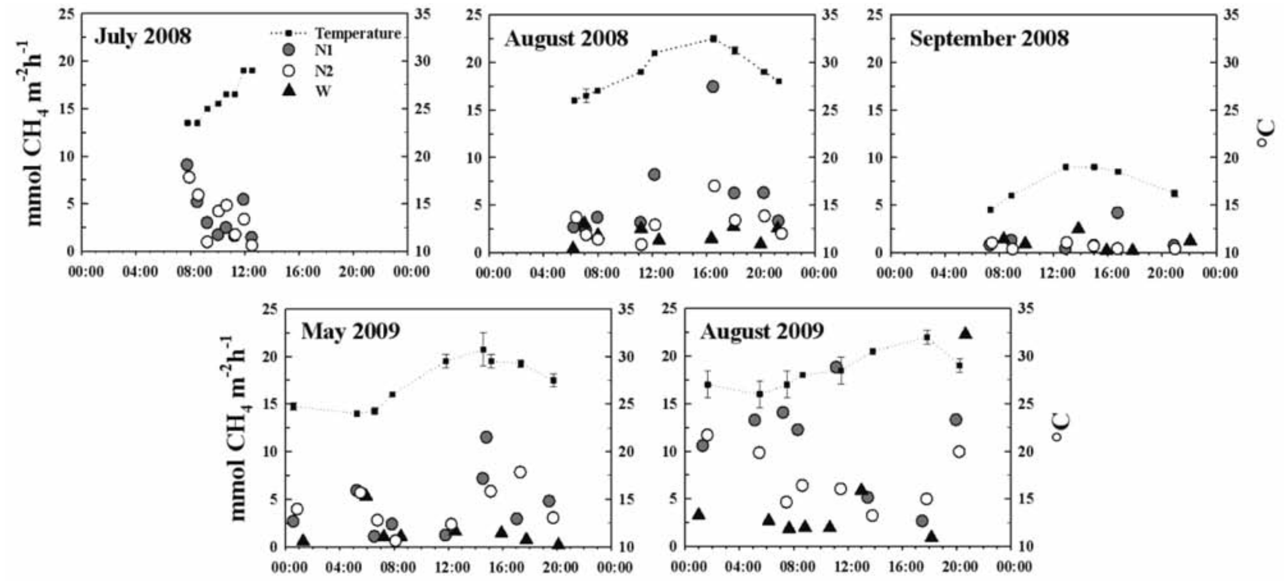

Fig. 3. Hourly CH_4 fluxes measured on a *N. lutea* stand (N1 and N2 stations) and on the free water surface (W station) during seasonal 24 h cycles. Note that the measurements performed in July 2008 refer only to daylight. Water temperature is reported on the dotted line and scatters and refers to measurements taken in correspondence of every measurement at the vegetated and at the free water station (bottom+surface samples, mean±SD, n=4).

Tab. 3. A three-way ANOVA was used to test the influence of the presence of the plants (Station), of the radiation intensity (Irrad) and of the water temperature (Temp) on the hourly CO_2 and CH4 fluxes (mmol m^{-2} h^{-1}) measured on a yellow waterlily stand (two stations, N1 and N2) and on the free water surface (one station, W). Factors were tested individually and by their interaction; level of statistical significance was set to α=0.05.

Factors	Hourly CO_2 fluxes *F* value	Pr	Hourly CH_4 fluxes *F* value	Pr
Station	$32.87_{2,97}$	<0.001	$1.78_{2,97}$	0.17
Irrad	$46.18_{1,97}$	<0.001	$0.07_{1,97}$	0.80
Temp	$4.95_{1,97}$	<0.05	$36.69_{1,97}$	<0.001
Station × Irrad	$7.06_{2,97}$	<0.01	$0.18_{2,97}$	0.84
Station × Temp	$0.54_{2,97}$	0.59	$1.39_{2,97}$	0.25
Irrad × Temp	$2.07_{1,97}$	0.15	$0.04_{1,97}$	0.83
Station × Irrad × Temp	$1.22_{2,97}$	0.30	$0.12_{2,97}$	0.89

only exception, while net CO_2 release was measured in the dark (Fig. 2). Hourly rates ranged between 23.1 and 10.8 mmol CO_2 m^{-2} h^{-1} and were negatively correlated with irradiance (R^2=0.49, p<0.01, n=78); as for the free water surface, CO_2 fluxes in vegetated stand were not correlated with dissolved CO_2 concentrations in the surface water. During the light period, daily CO_2 fluxes across the floating meadow varied between a net uptake of 172.4 mmol m^{-2} d^{-1} (May 2009, N1) and a release of 17.6 mmol m^{-2} d^{-1} (September 2008, N2) while during the dark period the CO_2 efflux was comprised between 16.2 and 110.0 mmol m^{-2} d^{-1} (Fig. 4). CO_2 fluxes resulted correlated with the plant biomass only during the light phase (R^2=0.13, p<0.01, n=63). Carbon fixation estimated from the CO_2 flux measurements within the *N. lutea* stand resulted in daily values ranging from 0.9±0.9 to 1.2±0.5 g C m^{-2} d^{-1} (Tab. 2), corresponding to 42.7±14.4 g C m^{-2} fixated during the period July-August 2008 and to 104.1±33.4 g C m^{-2} fixed during the period May-August 2009.

CH_4 fluxes showed similar daily patterns and ranges among all the sampled stations (Fig. 3 and Tab. 3). Hourly CH_4 efflux from the unvegetated and vegetated stations varied between 0.2 and 22.3 and between 0.2 and 9.0 mmol m^{-2} h^{-1}, respectively (Fig. 3).

At both vegetated and unvegetated areas, differences between CH_4 fluxes measured in the light and in the dark were not statistically significant; conversely, methane fluxes were positively correlated with water temperature

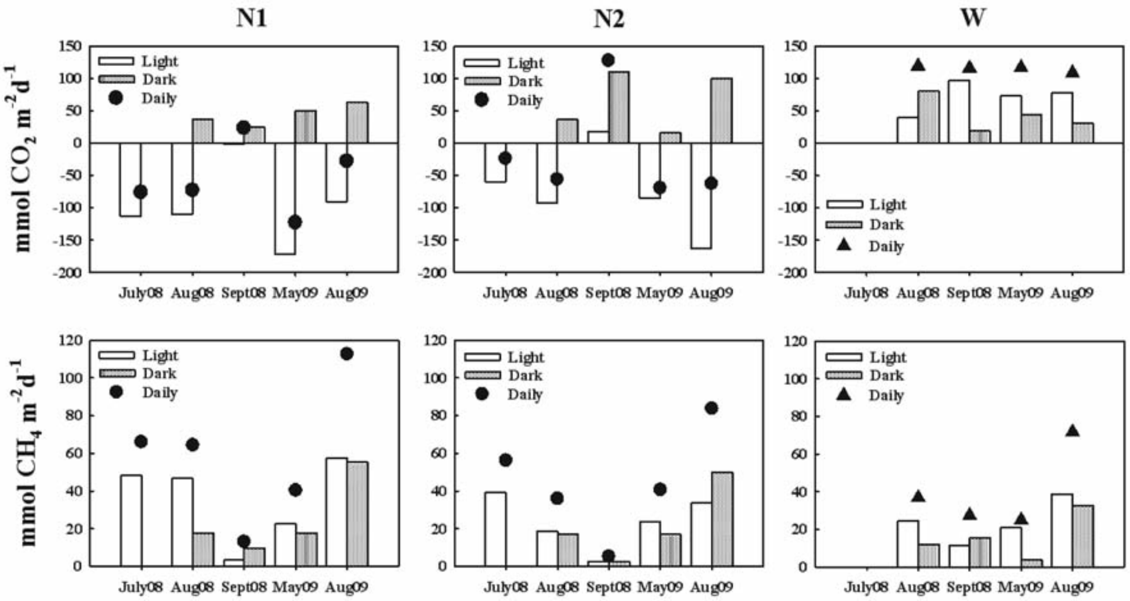

Fig. 4. Light (open bars), dark (shaded bars) and daily (scatters) CO_2 and CH_4 fluxes measured on a *N. lutea* stand (N1 and N2 stations) and on the free water surface (W station). Daily values referring to July 2008 on the vegetated stations were obtained by assuming a dark flux equal to that measured during the following campaign. Note the different scales between CO_2 and CH_4 flux representations.

(R^2=0.48, p<0.01, n=108) with a Q10 of 2.84. Within the *N. lutea* stand, CH_4 fluxes were positively correlated with both methane concentration in water (R^2=0.23, p<0.001, n=96) and the biomass of the yellow waterlily (R^2=0.21, p<0.001, n=79). Daily methane emissions followed a seasonal pattern, which was more evident at the vegetated stations than across the free water surface. CH_4 efflux in the *N. lutea* stand was minimum in autumn (5.2 mmol m^{-2} d^{-1}) and maximum in summer (112.6 mmol m^{-2} d^{-1}) while at the unvegetated water site daily emissions varied between 25.0 and 71.7 mmol m^{-2} d^{-1} (Fig. 4). During the vegetative period May-August 2009, CH_4 fluxes measured at the *N. lutea* stand resulted in a carbon release of 102.7±15.0 g C m^{-2}, while the free water surface released an amount of 71.6 g C m^{-2}.

DISCUSSION

Results of the present study only partially confirm our initial hypotheses. At the study site the well-developed *N. lutea* stand did not induce water anoxia and did not significantly affect the CO_2 and CH_4 concentrations compared to the unvegetated area. The general super-saturation of both gases resulted in large water-atmosphere gradients and effluxes, in particular during the dark phase. In the light, CO_2 uptake by the yellow waterlily leaves resulted in significant attenuation of the daily carbon dioxide release in the vegetated area compared to the plant-free water surface.

Despite the elevated LAI measured on *N. lutea* (0.8-1.5 m^2 m^{-2}, Tsuchiya 1991), the features of nymphaeid stands allow the water circulation and oxygenation under the floating meadow: due to slow rhizome elongation, they typically occupy only small portions of the basin they inhabit, mostly in the riparian zone. This is in contrast to free-floating plants communities, which commonly form dense stands and tend to invade the whole water surface (Janes et al. 1996; Bolpagni et al. 2007). Moreover, the heterophylly of this nymphaeid (*i.e.*, the coexisting presence of emergent, floating and submerged leaves) permits the light penetration down to the sediment surface, allowing photosynthetic activity in the shallow water column and in the sediment surface (Heslop-Harrison 1955; Zanaboni, and Pascoli 1988; Pełechaty, and Owsianny 2003).

We found that diffusive CH_4 efflux from the *N. lutea* meadow and the adjacent free water surface were not significantly different, while the amount of emitted CH_4 appreciably diverged from results reported for other sites within the boreal hemisphere (Tab. 4). The lack of difference between the vegetated and the free water stations might derive from factors as the elevated organic matter load in surface sediments, the high water temperatures and the low concentrations of electron acceptors as NO3$^-$, that favor intense methanogenesis (Longhi et al. 2008) (Tab. 4). The resulting CH_4 supersaturation in the water column maintains steep gradients with the atmosphere, which could mask the supposed contribution of aerenchyma transport to the overall CH_4 efflux. Elevated leaf production and photosynthetic rates could indeed favor both plant detritus supply and exudates release from roots, both

Tab. 4. Diffusive CH4 and CO2 fluxes (mmol m 2 d 1) measured on N. lutea stands and on the plant-free water surface at different locations during the vegetative period of the macrophyte. OM = organic matter content of sediments , measured as loss on ignition.

Site	Latitude	Elevation (m)	Depth (m)	Trophic state	Temp. (°C)	Leaf density (leaves m^{-2})	CH_4 flux ($mmol\ m^{-2}\ d^{-1}$) *N. lutea* meadow Mean	Range	Plant-free surface Mean	Range	CO_2 flux ($mmol\ m^{-2}\ d^{-1}$) *N. lutea* meadow Mean	Range	Plant-free surface Mean	Range	
Duck Lake, MI, USA	42°23’	283	2.0 (mean)	Eutrophic	-	10-20	19.7	-	6.7	-	-	-	-	-	Dacey & Klug (1979)
Red Rock Lake, CO, USA	40°04’	3095	1.0 (max)	25-31% OM	2-14	-	12.6	0-47	2.9	1-10	-	-	-	-	Smith & Lewis (1992)
Priest Pot, U.K.	54°22’	70	2.3 (mean)	23% OM	13-22	-	-	-	0.4	0.06-1.4	-	-	44	4-102	Casper et al. (2000)
Lake Kevätön, Finland	63°05’	91	2.3 (mean)	Hypereutrophic	2-17	2-18	-	-	-	-	-11	-100-10	-	-	Larmola et al. (2003)
Lake Kevätön, Finland	63°05’	91	2.3 (mean)	Hypereutrophic	2-17	-	-	-	3.2-5.1	0.3-12	-	-	14-15	-1.8-25	Huttunen et al. (2003)
Lake Vehmasjärvi, Finland	62°17’	116	3.9 (mean)	Eutrophic	2-17	-	-	-	0.1-0.4	0.1-0.4	-	-	11-28	3.9-32	Huttunen et al. (2003)
Lake Mäkijärvi, Finland	61°05’	88	3.4 (mean)	Oligotrophic	2-17	-	-	-	0.1-0.2	0.1-0.2	-	-	5.8-12	4.7-15	Huttunen et al. (2003)
Lake Ekojärvi, Finland	61°18’	109	0.6-1.0 (range)	21-39% OM	21-24	10-37	0.9	0.5-1.4	-	-	-	-	-	-	Kankaala et al. (2003)
Lake Vesijärvi, Finland	61°05’	110	0.3-0.7 (range)	44% OM	-	6	6-10	0-38	-	-	-	-	-	-	Kankaala et al. (2004)
Busatello Marsh, Italy	45°06’	11	0.6-0.9 (range)	30% OM	23-34	29-33	62-77	36-113	48.3	25-72	-66-75	-172-110	112.7	107-119	This study

of which fuel CH_4 production in the sediment (Schütz et al. 1989; Segers 1998; Wang, and Han 2005; Pełechaty 2007; Longhi et al. 2008). On the other hand, the downward O_2 transport to roots and associated ROL could promote CH_4 oxidation in the rhizosphere, with a lesser upward release of CH_4 through lacunal tissues (Große et al. 1996). This is still a controversial issue, as nymphaeid rhizome typically presents a barrier to avoid radial oxygen leaking, which canalizes longitudinal O_2 transport only towards the active-growing tips (Laing 1940; Smits et al. 1988; Seago et al. 2000; Colmer 2003). At any rate, the seasonal variations of methane efflux we measured resulted reliant on the water temperature and respiration processes more than to the yellow waterlily biomass variations. Consistently, the CH_4 fluxes measured at our site are greater than the emission range reported for *N. lutea* in other eutrophic shallow wetlands (Tab. 4), where rates of microbial processes are probably limited by low temperatures and both quantity and macromolecular composition of organic matter (Setälä, and Mäkelä 1991; Longhi et al. 2008).

In this study, repeated CO_2 flux measurements showed that the *N. lutea* stand considerably contributed to the carbon sequestration from the atmosphere. This is in agreement with previous findings for *N. lutea* (Wallace, and O’Hop 1985; Kok et al. 1990; Larmola et al. 2003). However, in this study average daily CO_2 fluxes were higher than those reported for another site (Larmola et al. 2003), the differences being attributable to different climatic conditions and length of the vegetative period. CO_2 efflux from the free water surface resulted comparable to sites located at the same latitude (Casper et al. 2000), while differed from other sites located at a higher latitude or elevation (Tab. 4).

The *N. lutea* biomass values we measured during summer were much higher than the maximum standing crop reported by Kankaala et al. (2004) and in the range of values (193-1751 g_{DW} m^{-2}) reported by Tsuchiya (1991). The leaf density and the net carbon fixation into biomass were higher than those reported by Wallace and O’Hop (1985), Setälä, and Mäkelä (1991) and Larmola et al. (2003). The leaf morphology was in agreement with those reported by Dacey (1981), Wallace and O’Hop (1985), Setälä, and Mäkelä (1991) and Tsuchiya (1991).

The net carbon fixation estimated through the leaf production resulted in values comparable to those reported by Wallace and O’Hop (1985) and Kok et al. (1990), and higher than those obtained from our CO_2 flux measurements, as also reported by Larmola et al. (2003). The gap between the two methods is presumably due to the fact that CO_2 fluxes measured with floating chambers are determined by the interaction of co-occurring processes (*i.e.*, CO_2 uptake by leaves, photo-respiration by plants, and CO_2 production and release from sediments and water column). In particular, the amount of CO_2 fixed by the floating leaves accounted only for the 30% of the aboveground biomass increase. This is a small amount when compared to other floating-leaved plants, whose carbon uptake mostly depends upon atmospheric CO_2 (Pierobon et al. 2010). In floating-leaved macrophytes, the carbon uptake from the adaxial side of the floating leaves derives from the atmospheric CO_2, while the growth of submerged leaves depends mainly on dissolved inorganic carbon fixation from the water column (Smits et al. 1988; Titus, and Sullivan 2001). Besides, in highly productive wetlands, where a great organic bulk is stored in the sediment, atmospheric CO_2 could become less important than internal inorganic carbon sources, which can contribute up to 85% of the photosynthetically fixed carbon (Dacey 1980; Dacey, and Klug 1982a).

The species composition and the inherent canopy structure of macrophyte meadows are recognized to regulate gas exchanges between water and atmosphere in wetlands and shallow lakes. Two opposite functional traits have been depicted. Submerged aquatic vegetation can control CH_4 production within and emission from the rhizosphere through both ROL and O_2 release in the water column (Caraco, and Cole 2002; Ribaudo et al. 2011). Conversely, free-floating plant communities are assumed to cause persistent anoxia along with elevated respiration and methanogenesis rates within the sediment (Pokorný, and Rejmánková 1983; Parr et al. 2002; Bolpagni et al. 2007). Results from this study indicate that the colonization of *N. lutea* might not have the same drastic effect reported for free-floating macrophytes in shallow aquatic ecosystems. At our study site, the yellow waterlily did not significantly alter the dissolved oxygen dynamics in the water column, yet a significant amount of carbon was fixed into biomass from both the water and the atmosphere. Moreover, the lack of difference in methane emission between the vegetated and the plant-free areas suggests that the general assumption that floating-leaved plants such as *N. lutea* are larger CH_4 sources than the plant-free water has to be carefully evaluated. In fact, the mineralization processes occurring during winter following the litter accumulation might produce a reversed outcome. Those processes would indeed be relevant at our latitude, where average water temperature allows the methano-genic activity to persist also during the winter time.

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