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Net primary production and seasonal CO_2 and CH_4 fluxes in a *Trapa natans* L. meadow

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ABSTRACT

The main hypothesis of this work is that Trapa natans *L. and similar floating leaved macrophytes are only temporary sinks of atmospheric carbon dioxide and that they favour water hypoxia and large methane efflux from sediment to the atmosphere, due to their shading effect and scarce ability to transfer oxygen to submerged tissues. For this purpose, from April to August 2005,* T. natans *production, dissolved O_2, CO_2 and CH_4 concentrations in the water column and CO_2 and CH_4 fluxes across the water-atmosphere interface were measured in an oxbow lake (Lanca di Po, Northern Italy) where a monospecific floating mat of water chestnut develops. Net primary production by* T. natans *was determined via biomass harvesting while gas fluxes were determined* via *short-term incubations of light and dark floating chambers. From July onwards, when the water surface of the oxbow lake was entirely colonized by the plant, the dense canopy resulted in a physical barrier for light and water reareation. As a consequence of sediment and plant respiration, persistent hypoxia and often anoxia, and CO_2 and CH_4 supersaturation occurred in the water column. Net primary production of* T. natans*, calculated at peak biomass, was 13.05 ± 0.32 mol CO_2 m^{-2}. The* T. natans *mat was a net sink for atmospheric CO_2 from mid June to mid August, with an uptake peak measured at the beginning of July (229 mmol m^{-2} d^{-1}); estimated net ecosystem metabolism was $\leq 10.09 \pm 1.90$ mol CO_2 m^{-2}. Contextually, during the vegetative period of* T. natans*, the oxbow lake was a net source of methane (9.52 ± 2.10 mol m^{-2}), and the resulting CH_4 to CO_2 flux ratio across the water-atmosphere interface was ≥ 0.94. The large methane release was probably due to the persistent hypoxia and anoxia induced by the* T. natans *meadow, which uncoupled methane production from methane oxidation.*

Key words: Trapa natans*, net primary production, gas flux, anoxia, CO_2, CH_4*

1. INTRODUCTION

Many oligotrophic lakes and most rivers are heterotrophic, acting as net sources of CO_2 and CH_4 to the atmosphere (Devol *et al*. 1987; Kling *et al*. 1992; Raymond *et al*. 1997; Duarte & Praire 2005; Walter *et al*. 2006). This is a consequence of allochtonous input of organic matter, resulting in microbial respiration largely prevailing over carbon fixation rates. In wetlands, where large pools of inorganic carbon are fixed by macrophytes, the evaluation of fixation rates and the understanding of processes regulating CO_2 and CH_4 fluxes have a high degree of uncertainty (Nieveen *et al.* 1998; Glenn *et al*. 2006; Hirota *et al*. 2006; Ding & Cai 2007; Saarnio *et al*. 2009; Zhou *et al*. 2009).

Wetland ecosystems generally display high potentials for carbon sequestration in plant biomass that result in large sedimentary pools of organic carbon and high rates of anaerobic microbial respiration (Bridges 1978; Wetzel 1990; Den Heyer & Kalff 1998; Whiting & Chanton 2001). In reduced wetland sediments, CH_4 production can potentially sustain elevated CH_4 fluxes to the water column and then to the atmosphere. Simultaneously, inhibition of methanogenesis or methane oxidation can occur in micro-oxic niches within the rhizosphere, thus avoiding the methane delivery into the atmosphere (King 1994; Roden & Wetzel 1996; van der Nat & Middelburg 1998; Brix *et al*. 2001). Wetlands of several types are net sinks for CO_2 and accumulate carbon at rates up to 3 g C m^{-2} d^{-1} (Trumbore *et al*. 1999; Hirota *et al*. 2006; Wetzel 2006; Bonneville *et al*. 2008; Zhou *et al*. 2009). Contrasting evidences feed the prediction that these ecosystems will rapidly turn from CO_2 sink into sources (Bubier *et al*. 2003; Bragazza *et al*. 2006; Strack *et al*. 2006). Despite usually high primary productivity in wetland environments (Wetzel 1990), the net balance between opposite CO_2 fluxes (from and to the atmosphere) often depends upon slight differences between community assimilation and respiration rates and varies both spatially and temporally (Bubier *et al*. 1999; Brix *et al*. 2001; De Vicente *et al*. 2006; Sand Jensen *et al*. 2007; Bonneville *et al*. 2008; Zhou *et al*. 2009).

Studies of primary productivity and CH_4 fluxes in shallow water environments mostly deal with submerged macrophytes or helophytes, while comparatively little research has addressed floating leaved species. In particular, their potential role as regulators of CO_2 and CH_4 exchanges at the sediment-water and water-atmosphere interfaces is still poorly understood. Portielje & Lijklema (1995) and Scheffer *et al*. (2003) suggested that increasing nutrient loadings may determine the irreversible displacement of submersed aquatic vegetation and the establishment of floating leaved communities. Pleustophytes reduce oxygen availability in the water column, with cascading impacts on trace gas dynamics (Wolek 1974; Pokorny & Rejmankova 1984; Caraco & Cole 2002; Caraco *et al*. 2006; Hummel & Findlay 2006; Goodwin *et al*. 2008).

Among pleustophytes, the common water chestnut *Trapa natans* L. is an annual species with emergent rosette leaves held up by inflated leafstalks and submersed feather leaves, with potential photosynthetic activity. This plant develops a complex system of stems, pseudo roots and roots weakly anchored to the substrate (Tsuchiya & Iwaki 1984; Hummel & Kiviat 2004). Previous studies showed that the dense *T. natans* canopy limits light penetration and promotes water column hypoxia (Caraco & Cole 2002; Bolpagni *et al*. 2006; Hummel & Findlay 2006; Goodwin *et al*. 2008).

The main hypothesis of this work is that *T. natans* is a temporary trap of carbon dioxide but simultaneously, due to the shading effect of its canopy, induced anoxia and scarce ability to transfer oxygen to submerged tissues (Caraco & Cole 2002), it promotes methane production and release. To this purpose, net primary production of *T. natans* (biomass harvesting) and fluxes of CO_2 and CH_4 across the floating meadow-atmosphere interface (static chambers incubation) were measured in an oxbow lake during the growth period of the pleustophyte.

2. MATERIALS AND METHODS

2.1. Study site

All measurements were performed in an eutrophic oxbow lake (Lanca di Po, 45°1'N, 10°1'E), which is an old meander of the Po River (Northern Italy). The Lanca di Po covers an area of 0.07 km^2 and has an average depth of 1 m, ranging from 0.2 to 3 m. The lake is fed by groundwater and, occasionally, by the Po River during spring or autumn floods; which occurs approximately every 3 to 5 years when discharge exceeds 4000 $m^3\ s^{-1}$.

The seasonal succession of the primary producer communities consists of a spring phytoplankton bloom, which is followed by the development of a dense meadow of *T. natans*, which colonizes the whole basin (Bolpagni *et al.* 2006). Nine sampling campaigns were performed on 24 April, 3, 19 and 30 May, 16 June, 7 and 28 July, 10 and 22 August 2005.

Water and biomass were sampled, and CO_2 and CH_4 exchanges at the water-atmosphere interface were measured in an undisturbed station located in the central portion of the oxbow lake, within an homogeneous stand of *T. natans*. Samples collection and incubations were performed from a small platform suspended above the water surface to avoid sediment and water disturbance.

2.2. Water characteristics

Temperature, conductivity at 25 °C and pH were measured *in situ* with a portable multiple probe (YSI Instrument, mod. 556). Water was sampled with a syringe close to the water-atmosphere and at the water-sediment interfaces. One sample was immediately fixed with Winkler reagents for oxygen determination (APHA 1981). An aliquot was poisoned with 100 µL of $HgCl_2$ and total inorganic carbon (TCO_2) was measured with Gran titration (Anderson 1986). Dissolved CO_2 concentration and saturation values were calculated according to Lewis & Wallace (1998) from pH, TCO_2, temperature and conductivity data. A water aliquot was transferred in an exetainer (Labco UK), and was analysed for CH_4 with a gascromatograph (Fisons Instruments, 9000 GC series) equipped with a FID detector.

2.3. Trapa natans *biomass*

On each sampling date *T. natans* was harvested in triplicate with a 0.25 m^2 hoop randomly positioned on the meadow. Floating and submersed portions of the living biomass were collected; while the dead fraction was not considered. Care was taken to extrude all the tiny roots and eventual seeds from the fluffy sediments. Once in the laboratory, plants were gently washed to remove periphyton. Biomass from each replicate was subdivided into leaves, petioles, stems, seeds, roots and pseudo roots for subsequent determination of fresh weight. Dry weight was then measured after 48 hours at 60 °C. The total plant biomass and the relative contribution of the different components were then calculated.

Biomass specific *T. natans* growth (daily variation in biomass normalized by mean biomass between two successive samplings, BSG) was calculated according to equation 1 (Kemp *et al.* 1986):

$$BSG = \frac{B_{t+n} - B_t}{(B_{t+n} + B_t) \times 0.5 \times n} \qquad (1$$

where *BSG* (mg $g_{DW}^{-1}\ d^{-1}$) is the *T. natans* biomass specific growth, B_t is the biomass at time *t* and B_{t+n} is the biomass after *n* days.

Net changes of *T. natans* biomass *versus* time were modeled with an exponential polynomial function (equation 2) that is a modified Ricker equation, generally used in studies of population dynamics (Ricker 1958):

$$B = -k \times (x - a) \times e^{J \times (x - a)} \qquad (2$$

where *B* ($g_{DW}\ m^{-2}$) is the *T. natans* biomass at time *x* (days); the parameter *a* is the time span of *T. natans* life cycle, fixed to 148 days, from the beginning of April to the end of August; *J* (0.0314 ± 0.0016) is a constant term that determines the curvature of the net biomass pattern and can be calculated from the point of biomass peak and *k* (0.0435 ± 0.0034) is a constant term that affects the slope and the magnitude of growth evolution. *k* and *J* coefficients have no biological interpretation; once fixed the time span of the function, *J* is determined and *k* optimum value is found using the maximum likelihood method. One thousand of randomly generated curves (statistical package *R*, Version 2.8.1) were used to associate a standard error to the generated biomass values and to the integrated overall biomass. The model was then used to simulate the carbon fluxes (fixation and loss) through the *T. natans* stand.

2.4. CO_2 and CH_4 flux measurements

The closed chamber technique (Crill *et al*. 1988) was applied for measurements of CO_2 and CH_4 fluxes under daylight and under night conditions. On each sampling date, 6 replicated series of measurements were performed, 3 at midday and 3 before sunrise. Each series of measurements consisted in a short-term incubation of 8 floating plexiglass chambers (internal diameter 38 cm, total volume 3.5 L). Four chambers were positioned above *T. natans* rosettes and four chambers were positioned above the free water surface, after gently removing the plants. During the sampling period all measurements were performed at the same site and, for each parameter and each sampling date, averages were calculated on 12 replicates.

All chambers were equipped with a gas sampling port, a gas compensation bag, a small 12 V fan for mixing the internal atmosphere and a sensor for monitoring the chamber temperature and humidity (Oregon Scientific remote sensor, mod THGR228N). Light attenuation due to plexiglass chambers was negligible as measured PAR reduction was always <10%. Atmospheric temperature and humidity were measured with another sensor positioned above the plants mat. Pre-incubations were performed to establish the incubation time required for reliable flux measurements and to avoid excessive increases of temperature and humidity within floating chambers. Gas was sampled at time zero and after 0.5, 1.5, 2.5 and 6 minutes; gas fluxes were calculated in the linear portion of the regression of gas concentrations versus time and regressions with a determination coefficient <0.9 (i.e., due to gas bubbling) were discarded. Gas samples (9 mL), were withdrawn with a syringe and immediately transferred into gas tight tubes (Terumo Venoject, Belgium). Within 24 hours from sampling, CO_2 was analyzed with a thermo electron trace gascromatograph with a TCD detector; CH_4 was determined with a gascromatograph (Fisons Instruments, 9000 GC series), equipped with a FID detector for CH_4 (Bodelier *et al*. 2000). During each series of incubation light intensity was measured with a portable spectroquantophotometer (Delta OHM, mod HD9021).

In this work positive CO_2 or CH_4 fluxes are intended as emissions to the atmosphere while negative fluxes are directed toward either the water column or the water and *T. natans* meadow (Morison *et al*. 2000).

Hourly CO_2 fluxes measured during daylight represented the maximum net ecosystem production (NEP, mmol C m^{-2} h^{-1}) as they were measured at midday, fluxes measured in the nighttime represented the ecosystem respiration (RE, mmol C m^{-2} h^{-1}). Daily NEP and night RE were then estimated by multiplying hourly NEP and RE rates by the corresponding number of daylight and night hours of the sampling period. The algebraic sum of daylight NEP and nighttime RE gave the net daily ecosystem metabolism (NEMdaily = NEPhourly×hlight + REhourly×hdark). Hourly Gross Ecosystem Production (GEP, mmol C m^{-2} h^{-1}) was calculated as the algebraic sum of NEP and RE (Shaver *et al*. 1998; Caraco & Cole 2002; Howarth *et al*. 1996).

All calculations were based on the assumption that both hourly NEP and RE rates were constant during daylight and nighttime, respectively. Although this is a reasonable assumption for dark rates (Bolpagni *et al*. 2007) it is not for rates in the light, as measurements were performed at the saturating light intensity. As a result, the reported daily and seasonal NEP, NEM and GEP rates must be considered as maximum rates. Daily balances of CH_4 were calculated similarly and underwent the same assumptions.

2.5. Statistical analyses

Pearson's correlation and linear regression between measured fluxes and physico-chemical features of the water were computed with standard statistical packages (SPSS, Ver. 13.0). The statistical significance of r was tested using a *t*-test. Gas flux data were checked for normality and homogeneity of variance, and log-transformed when appropriate; differences between fluxes were tested by means of three-way analysis of variance (ANOVA) with sampling date, presence of *T. natans* and light as factors.

3. RESULTS

3.1. Water characteristics

In 2005, spring and summer were particularly dry, without river floods and with little input of water into the oxbow lake. For these reasons and as a consequence of evapotranspiration, at the sampling site water depth decreased from 0.65 (May) to 0.35 m (late August) and conductivity increased from 303 to 899 µS cm^{-1}.

From April to August, increasing water temperatures and the development of a dense canopy of *T. natans* led to a marked decrease of dissolved O_2 concentration. In July and August, coinciding with the biomass peak, bottom water hypoxia established, with undetectable concentrations or O_2 saturation systematically below 5% (Tab. 1). CO_2 and CH_4 concentrations exhibited opposite patterns and accumulated in the water column over the super-saturation levels (Tab. 1). pH changes were also measured, from close to 7 in the bottom water at night to 8.70 in surface layers during daylight.

3.2. Seasonal dynamics of T. natans *biomass*

Trapa natans rosettes emerged in late April and spread over the whole basin forming an homogeneous

Tab. 1. Water column temperature and dissolved gas concentrations measured from 24 April to 22 August 2005 within the *T. natans* meadow, in the central portion of the oxbow lake where gas flux measurements were performed. Values refer to measurements performed at midday (Light) and before sunrise (Dark) close to the water-atmosphere (S) and sediment-water interfaces (B). Oxygen deficit and CO_2 and CH_4 supersaturation can be appreciated considering that in the range of measured temperatures, 100% saturation is comprised between 239 and 300 µM for O_2, 9-12 µM for CO_2 and 2.6-3.3 nM for CH_4 (Sander 1999).

Date	Temperature (°C)		O_2 (µM)		CO_2 (µM)		CH_4 (µM)	
	Light S-B	Dark S-B	Light S-B	Dark S-B	Light S-B	Dark S-B	Light S-B	Dark S-B
24/04	23.2-21.4	20.2-19.8	378-220	370-190	7-29	10-43	0-0	0-0
03/05	24.5-21.9	21.6-20.6	367-182	360-143	9-105	9-162	0-0	0-0
19/05	25.0-22.0	22.0-22.0	389-130	367-108	11-303	11-243	0-1	0-3
30/05	32.0-30.0	22.5-20.6	332-59	41-14	60-317	261-279	4-17	6-9
16/06	32.0-30.0	25.1-24.6	216-57	19-13	14-285	160-282	5-10	5-19
07/07	24.0-24.0	24.0-23.0	36-9	3-0	35-422	620-1056	22-51	35-188
28/07	30.7-28.5	28.4-27.1	215-0	6-0	1540-2081	1792-1610	122-241	188-424
10/08	28.1-24.4	27.3-23.9	230-0	4-0	1230-1570	1480-1583	103-176	167-230
22/08	27.2-20.1	22.8-20.9	242-7	6-0	370-717	720-791	93-228	142-198

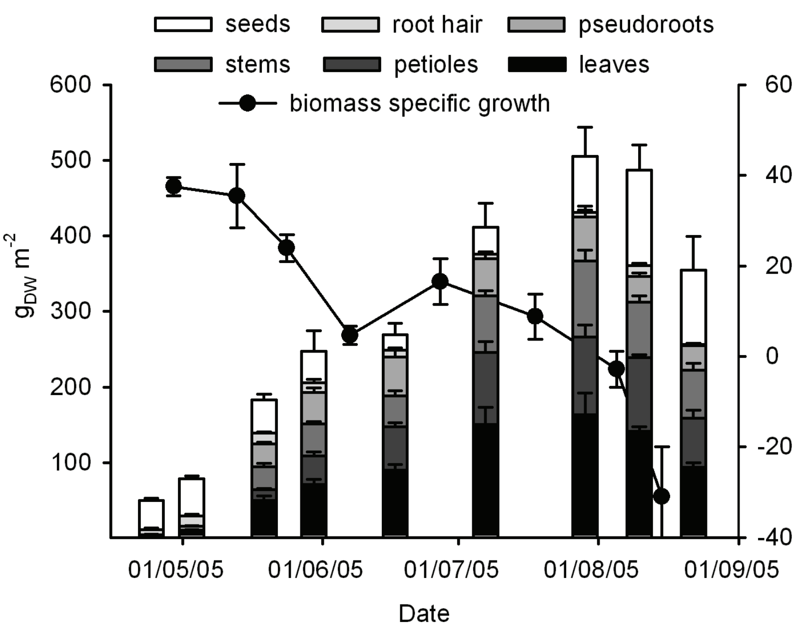


Fig. 1. Bars represent *T. natans* biomass ($g_{DW}m^{-2}$) and its allocation into different plant components from 24 April to 22 August 2005. Dots represent the biomass specific growth (daily variation in biomass normalized by mean biomass between two successive samplings, equation 1, mg $g_{DW}^{-1}d^{-1}$) of *T. natans*. Average values (n = 3) ± standard deviations are reported (see the text for major details).

meadow in nearly one month. The total biomass increased markedly from 47.1 ± 7 g m^{-2} to 504.8 ± 90.6 g m^{-2} as dry weight in July (Fig. 1). A 30% biomass loss was measured one month later, suggesting a fast decay phase which was followed by the meadow collapse.

Most submerged biomass was composed of seeds, stems, roots and pseudoroots, while after the meadow formation floating rosette (leaves and petioles) prevailed, accounting for up to 78% of the total biomass. Along with the biomass increase, biomass specific growth rate decreased progressively from 37.0 ± 2.3 mg g^{-1} d^{-1}, during the submerged phase, to -30.9 ± 9.9 mg g^{-1} d^{-1}, after the onset of decay (Fig. 1). The best fit of biomass versus time (equation 2) accurately described the seasonal dynamics of *T. natans* biomass (R^2 = 0.98, p <0.01) for both growth and decay phases (Fig. 2). Net rates of *T. natans* biomass accumulation, calculated with the first derivative of equation 2, varied from an initial minimum of 2.1 g m^{-2} d^{-1} (24/04/05) to 5.9 g m^{-2} d^{-1} (01/07/05). Assuming a conservative carbon content of 35% (Fernandez-Alàez *et al*. 1999; Rejmankova 2005) the resulting net inorganic carbon uptake by *T. natans* peaked on 1 July at -172 mmol CO_2 m^{-2} d^{-1} and progressively decreased afterwards. Over the study period, net primary production by *T. natans* was estimated in 13.05 ± 0.32 mol CO_2 m^{-2}.

3.3. CO_2 fluxes

Maximum irradiance during chamber incubation varied between 700 and 800 µE m^{-2} s^{-1} until early May.

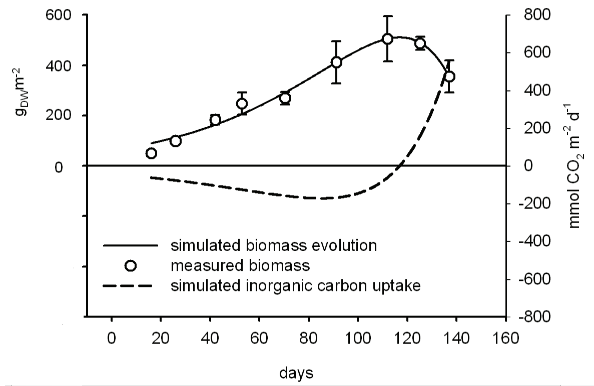


Fig. 2. Exponential polynomial growth function (equation 2) fitted to *T. natans* biomass data. Dashed line is the simulated inorganic carbon uptake to sustain *T. natans* growth (see the text for major details).

Afterwards it increased, ranging from 1300 to 2100 µE m^{-2} s^{-1}. In the floating chambers without *T. natans* rosettes, CO_2 fluxes were directed towards the atmosphere on all dates and both in the light and in the dark incubations. CO_2 efflux rates were between 0.82 ± 0.50 in the light on 24 April and 28.12 ± 2.40 mmol m^{-2} h^{-1} in the dark on 28 July (Fig. 3). On average, dark fluxes were greater than light fluxes, resulting in RE:NEP >4 on 27 July. In the light chambers, the CO_2 efflux to the atmosphere was likely attenuated by photosynthesis of below-surface plant biomass and associated epiphytes. Dark CO_2 fluxes were positively correlated with both surface water temperature (r = 0.93, n = 108, P <0.01) and CO_2 concentration in the surface water (r = 0.95, n = 108, P <0.01), while correlations were not significant for the light incubations. GEP, calculated from light and dark rates assuming constant respiration rates during the 24 hours, varied between -0.06 ± 0.98 (03/05/05) and -22.22 ± 3.50 (28/07/05) mmol CO_2 m^{-2} h^{-1}. Overall, in the considered period, the NEM for the water column devoid of floating rosettes was heterotrophic, with an estimated CO_2 efflux to the atmosphere of 20.42 ± 3.75 mol m^{-2} (Figs 4 and 5).

On each sampling date, in the light incubations, CO_2 fluxes across the *T. natans* mat were significantly different from those measured across the free water surface (ANOVA, P <0.01). In the daylight, the meadow was an atmospheric CO_2 sink, with maximum net uptake rates ranging from -0.20 ± 0.30 to -19.13 ± 8.91 mmol m^{-2} h^{-1}. In the nighttime, the meadow was a net source of

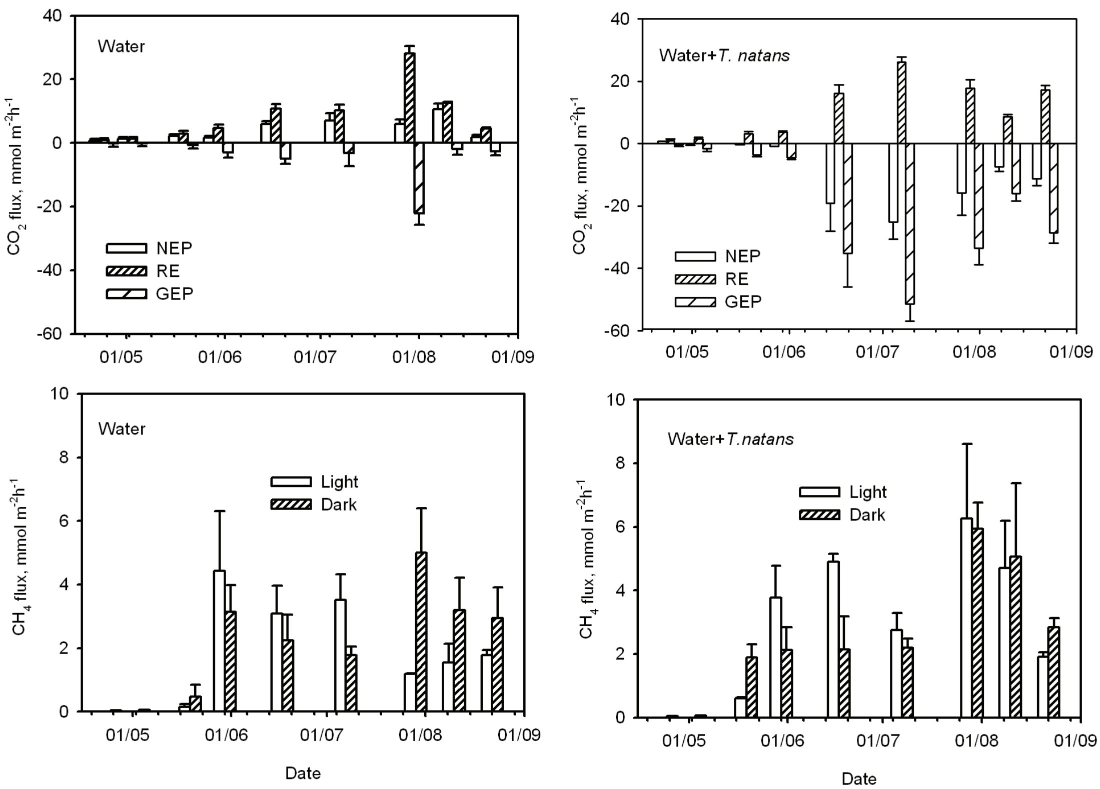
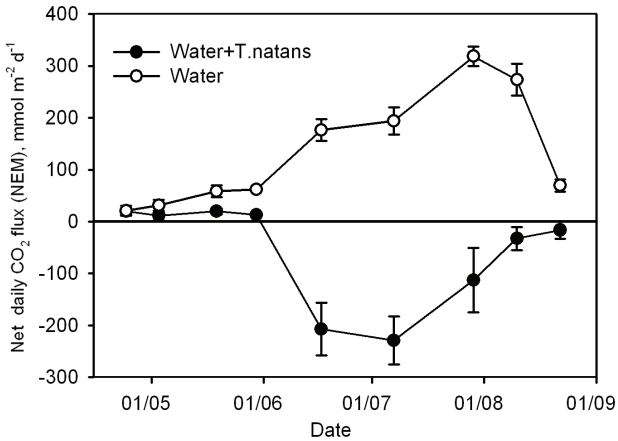


Fig. 3. Upper graphs. Seasonal maximum net (NEP) and gross (GEP) ecosystem production, and ecosystem respiration (RE) estimated from CO_2 fluxes across the free water-atmosphere interface and across the *T. natans* meadow. Negative CO_2 fluxes are directed from the atmosphere to the water or to *T. natans*, while positive fluxes are directed to the atmosphere. Average values (n = 12) ± standard errors are reported. Lower graphs. Seasonal fluxes of CH_4 across the free water-atmosphere interface and across the *T. natans* meadow measured in the light and in the dark. Average values (n = 12) ± standard errors are reported.



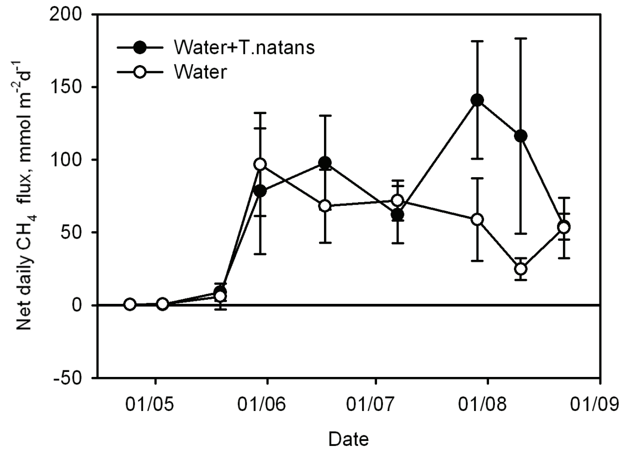


Fig. 4. Daily CO_2 (NEM) and CH_4 net fluxes across the free water-atmosphere interface and across the *T. natans* meadow. Values were obtained by multiplying light and dark hourly fluxes by the number of daylight and dark hours of each sampling date and summing up the obtained amounts. Average values (n = 12) ± standard errors are reported.

CO_2, with emissions ranging from 0.94 ± 0.61 to 26.21 ± 1.60 mmol m^{-2} h^{-1}. CO_2 fluxes measured in the light and in the dark were not correlated with surface water temperatures, dissolved carbon dioxide concentrations and *T. natans* biomass. In the floating mat, GEP varied between -0.22 ± 0.65 and -51.84 ± 5.61 mmol m^{-2} h^{-1} (Fig. 3). The daily NEM of the *T. natans* was slightly heterotrophic before the meadow formation; afterwards it became net autotrophic, until the onset of the decay phase (Fig. 4). Over the considered period, the water chestnut meadow was a net CO_2 sink with an overall maximum uptake estimated in -10.09 ± 1.90 mol m^{-2} (Fig. 5).

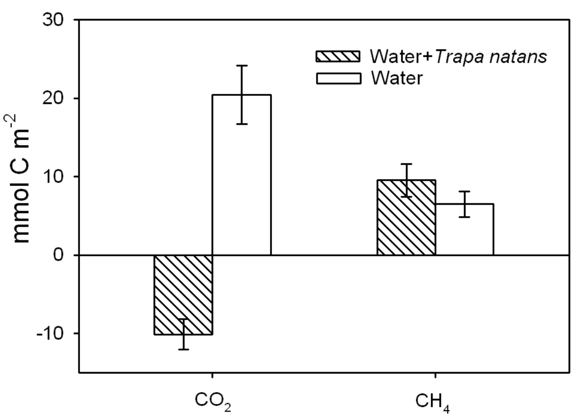


Fig. 5. Balances of CO_2 and CH_4 fluxes across the free water-atmosphere interface and across the *T. natans* meadow. Balances refer to the whole considered period (24 April – 22 August).

3.4. CH_4 fluxes

Methane fluxes were clearly affected by the seasonal cycle of *T. natans*. Methane emission from water to the atmosphere was negligible before rosette emersion. After the meadow formation all methane fluxes were towards the atmosphere without a clear seasonal pattern (Fig. 3). The greatest CH_4 emission rate coincided with the plant biomass peak, highest dissolved CH_4 concentration in the water and persistent anoxia in the water column. Fluxes of CH_4 towards the atmosphere were significantly higher across the *T. natans* stands than across the free water surface (ANOVA, P <0.01), although significant interaction terms suggested that differences depended on sampling dates and light (Fig. 4). Overall, during the sampling period, the meadow was a net CH_4 source with an estimated flux to the atmosphere of 9.52 ± 2.10 mol m^{-2}; while CH_4 release from the water surface devoid of rosettes was estimated in 6.49 ± 1.62 mol m^{-2} (Fig. 5).

4. DISCUSSION

4.1. Engineer species, pleustophytes and anoxia

This study supports the evidence that pleustophytes as *T. natans* act as engineer species, inducing structural changes within the ecosystems that they colonize (Jones *et al*. 1997). At the Lanca di Po, *T. natans* had a large pool of seeds in surface sediments and developed a monospecific meadow that colonised the whole oxbow lake outcompeting any other primary production by submerged forms (Groth *et al*. 1996; Takamura *et al.* 2003; Bolpagni *et al*. 2006).

The huge development of floating rosettes by *T. natans* is a typical strategy of invasive pleustophyte species, which exclude other primary producers through the competition for light (Caraco & Cole 2002; Scheffer *et al.* 2003; Goodwin *et al.* 2008). Biomass peaks up to 500 g_{DW} m^{-2} for this species are common in temperate regions, where temperature and light are not limiting factors during the growth season (Galanti & Topa Esposito 1996). Therefore, life cycle, metabolic traits and adaptive strategies of *T. natans* have to be evaluated when considering pathways and fate of inorganic carbon in ecosystems which are dominated by this growth form.

Together with plant development, hypoxia and reducing conditions became established in surface sediments and in the water column, as vascular plants with floating leaves deliver oxygen directly into the atmosphere, while the fixed carbon is for the most part retained within the aquatic ecosystem (Pokorny & Rejmankova 1984; Caraco & Cole 2002; Strayer *et al*. 2003; Bolpagni *et al*. 2007; Goodwin *et al*. 2008). Hypoxia or even anoxia have been reported for other wetlands and shallow lakes with *T. natans* stands (Caraco & Cole 2002; Takamura *et al*. 2003; Hummet & Findlay 2006; Goodwin *et al*. 2008) and in laboratory experiments (Tsuchiya & Iwakuma 1993).

A strong influence of primary producers on water chemistry has been reported for a number of shallow eutrophic ponds, where pleustonic species as *Lemna minor*, *L. gibba*, *Spirodela polyrhiza* or the floating fern *Salvinia natans* are dominant (Landolt 1986; Janse & Van Puijenbroek 1998; Jampeetong & Brix 2009). Persistent anoxia induced by these plants during warmest months favour the exhaustion of geochemical buffers within sediments (i.e., the ferric iron pool) and the release of nutrients (NH_4^+ and PO_4^{3-}) to the water column, with a positive feedback on the hypetrophic status of the ecosystem. For this reason, there is growing concern that aquatic environments exhibiting prolonged anoxia are then more prone to hypereutrophic conditions.

With respect to other species which evolve lacunal tissues and internal oxygen transport, as *Nuphar luteum*, *Nymphaea alba*, *Nelumbo nucifera* and *Nymphoides peltata*, *T. natans* has a limited capacity to transfer oxygen to submerged tissues (Pokorny & Rejmankova 1984; Caraco & Cole 2002). Therefore, one can assume that within *T. natans* stands, oxygen transport is not sufficient to compensate for oxygen consumption. In other words, this kind of meadow selects hypoxic/anoxic conditions, which in turn tend to support reducing metabolic processes. Here, high CH_4 effluxes compared to CO_2 fixation rates are basically coupled to anoxia induced by floating mats and the absence of significant methane oxidation.

Even if this study is limited to one species and one investigated site, obtained results should be carefully considered as floating leaved species are expanding in lenthic eutrophic environments, e.g. Lemnaceae and the aquatic fern *S. natans*.

4.2. T. natans *net primary production and net ecosystem metabolism*

In the present work, we estimated net primary production by *T. natans* with the biomass harvesting method and we simultaneously estimated net ecosystem metabolism by means of static chambers incubation. Both methods gave similar rates, which were 13.05 ± 0.32 mol m^{-2} from the harvesting method and 10.09 ± 1.90 mol m^{-2} from chamber incubations; but these two estimates should be considered with caution. The net primary production calculated from biomass harvesting underestimates true carbon fixation by *T. natans* as it does not include plant material lost by death and shedding, which can account for up to 50% of the fixed carbon (Tsuchiya & Iwaki 1984). Nevertheless, it is among the highest measured in analogous studies. Higher values, up to 46 mol C m^{-2} y^{-1} (Brix *et al*. 2001) and 22 mol C m^{-2} y^{-1} (Bonneville *et al.* 2008), were found for *P. australis*, which indeed attained a standing biomass many folds higher than that of *T. natans* in our study site. Measurements of CO_2 uptake with static chambers were made only at midday, when photosynthesis saturation likely occurred, and thus reported NEP and NEM are overestimates of true rates and should be considered as maximum rates.

By comparing the evolution of carbon uptake by *T. natans*, simulated from biomass data, and measured CO_2 fluxes across the vegetated water surface, the study period can be divided in three phases. In the early growth phase of *T. natans* midday incubations did not detect any significant CO_2 uptake from the atmosphere, while biomass evolution indicated a significant carbon fixation. This could be explained by uptake of carbon from the submersed parts of the plant, which likely had some photosynthetic activity (Goodwin *et al*. 2008). In summer, at the biomass peak, most of the CO_2 fixed by the plant was probably supplied by the atmosphere and rates estimated with the two methods showed a good agreement. In the senescence phase, biomass data indicated that CO_2 was regenerated and delivered back to the atmosphere. In this latter phase, a residual photosynthetic activity was detected also within the decaying mat, but the amount of fixed carbon was much less than the lost biomass.

Overall, it is likely that the Lanca di Po behaved as a sink for atmospheric CO_2 only for a short period, from June to the end of July, which coincided with the maximum development of the *T. natans* meadow. For the rest of the study period the oxbow lake was a source of CO_2 to the atmosphere. Analogous outcomes were reported for common reed wetlands, where the CO_2 stored by the reed stands was mainly fixed in June and July, while the cumulative emission during non-growing season was about 6 folds higher than the fixed quota (Zhou *et al*. 2009). Similar results were found in an eutrophic lake with *N. luteum* and *Phragmites australis* (Larmola *et al*. 2003), in peatlands with *Sphagnum* and *Carex* species (Glenn *et al*. 2006), and in a marsh with *Typha latifolia* (Bonneville *et al*. 2008), where macrophytes took up CO_2 only over to 2-4 months, generally comprised between May and September, while ecosystem respiration drove CO_2 fluxes for the rest of the year.

4.3. Greenhouse gas emission from T. natans *stands*

In the four month period of the present study, the development of the *T. natans* meadow resulted in the progressive depletion of dissolved oxygen, along with CO_2 and CH_4 supersaturation. The accumulation of CO_2 was probably due to very high respiration rates both at the sediment surface and within the water column, where senescent macrophyte accumulated and decomposed. The accumulation of CH_4 was likely a consequence of exhaustion of other electron acceptors, as O_2 or NO_3^-, elevated organic matter availability and very limited oxidation, due to hypoxia and even anoxia. Methane efflux from sediment or from the water column is generally a small fraction of the produced methane, due to elevated oxidation rates (Casper *et al*. 2000; Heilman & Carlton 2001). Brix *et al*. (2001) estimated that up to 76% of the methane produced in the sediment was reoxidised within the rhizosphere of *P. australis*. *T. natans* and similar pleustonic species can also act as physical barriers, which limit reareation and gas release, thus favoring both CO_2 and CH_4 accumulation in the water column beneath. However, in our site, nonetheless the supersaturation the CO_2 balance in the *T. natans* mat was negative; while CH_4 was net released.

Ding & Cai (2007) reviewed factors controlling CH_4 emission from different macrophyte communities. Methane release was mostly controlled by temperature and depth of standing water. Diel variation in CH_4 emission were controlled by irradiance and depended on macrophyte specific stomatal conductance, with higher release from emergent compared to submerged species. Emission rates measured during the vegetative period were extremely variable, from 0.3 mmol CH_4 m^{-2} d^{-1} in peatland with *Ranunculus trichophyllus* to 58.9 mmol CH_4 m^{-2} d^{-1} in freshwater marshes with *P. australis*. Such rates, integrated for the vegetative period, gave seasonal methane emission that were significantly lower than that reported in the present study. Rates falling in this range were also reported by Saarnio *et al*. (2009) for European wetlands and rivers.

The net global warming potential associated to seasonal CO_2 and CH_4 exchanges in the Lanca di Po can be roughly estimated using the carbon sink/source function proposed by Brix *et al*. (2001), that considers the relative radiative forcing of these two greenhouse gases along different temporal scales. Due to the much higher global warming potential of methane compared to carbon dioxide, a natural environment should exhibit ratios between CO_2 fixed and CH_4 released ≥ 20 in order to behave as a net greenhouse gas sink. Only few studies report CH_4 to CO_2 flux ratios, ranging between 0.05 and 0.13 for many macrophyte and helophyte species (Whiting & Chanton 1997; Brix *et al*. 2001; Zhou *et al*. 2009). Considering the relatively faster decay rate of methane in the atmosphere, wetland with ratios comprised between 0.05 and 0.13 are temporary sources of greenhouse gases for periods estimated in 60-120 years, after which they then turn into net greenhouse gas sinks. In our study site, the molar ratio between CH_4 emitted and CO_2 fixed, calculated for the study period with static chambers, was ≥ 0.94 as NEM was likely overestimated. This means that the two opposite fluxes were nearly balanced and that even in a long term perspective (>500 years) the greenhouse effect of released methane would prevail over the beneficial effect of CO_2 sequestration. Again, these preliminary results should be interpreted with caution, but they provide evidences that pleustonic communities uncouple methane production and oxidation and promote the release of this greenhouse gas to the atmosphere.

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