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WETLANDS BIODIVERSITY AND PROCESSES

Do oxic–anoxic transitions constrain organic matter mineralization in eutrophic freshwater wetlands?

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Abstract This study aims at investigating decomposition processes in wetlands that are daily or seasonally exposed to intermittent oxic and anoxic conditions. We hypothesized that in wetland ecosystems where anoxia regularly establishes, decomposition rates are not affected by oxygen shortage, especially when nitrates are available. Monitoring and experiments were performed from December 2003 to January 2005 in one of the widest (81 ha) freshwater wetlands in the Po river floodplain (Natural Reserve Paludi del Busatello, Italy). Intact sediment cores were sampled on a seasonal basis. Sediment–water fluxes of oxygen, dissolved inorganic carbon, methane, and inorganic nitrogen were determined under oxic and anoxic conditions. Oxic–anoxic transitions always resulted in enhanced ammonium, dissolved inorganic carbon, and methane effluxes. At high temperatures, the methane release from sediments was inversely related to both nitrate concentrations and uptake. Likely, nitrate can compensate for the oxygen deficiency while maintaining an oxidative metabolism, either supporting microbial decomposition as an electron acceptor or stimulating the oxidation of the byproducts of the anaerobic metabolism, e.g., methane. This is a key point as the number of temperate wetlands with concurrent nitrate pollution and oxygen shortage is increasing throughout the world.

Keywords Eutrophic wetlands · Oxygen availability · Nitrate · Organic matter mineralization

Introduction

An important part of the oxidation of organic matter (OM) in freshwater ecosystems takes place in surface sediments through aerobic microbial processes because oxygen is the energetically most favorable electron acceptor (Sørensen et al., 1979). However, oxygen penetration in the sediments is generally very low and restricted to the uppermost millimeters (Revsbech et al., 1980). In the underlying anoxic horizons, OM is decomposed through anaerobic processes, with electron acceptors other than oxygen. Here, aerobic OM decomposition is also contrasted by the oxygen consumption from the reoxidation of the byproducts of the microbial anaerobic metabolism (e.g., NH_4^+, Fe^{2+}, Mn^{2+}, S^{2-}, and CH_4), which can attain up to 75% of the total sediment oxygen demand (Sweerts et al., 1991). Therefore, in many aquatic ecosystems a major percentage of OM may be processed through anaerobic pathways (Kristensen & Blackburn, 1987).

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The degradation of sedimentary OM is also controlled by its macromolecular structure and elemental composition. At low oxygen concentrations, decomposition of refractory OM is slower than under normoxic conditions, while degradation kinetics and efficiency of labile OM are less affected by oxygen availability (Kristensen et al., 1995; Sun et al., 1997). However, the dependence of the OM biodegradability on the sediment oxygenation degree depends also upon other factors including the presence of alternative electron acceptors, e.g., nitrates, the redox condition oscillation, the presence of macrophytes, and the bioturbation activity by benthic fauna (Hulthe et al., 1998; Bastviken et al., 2004; Maerki et al., 2009).

The paradigm that OM decomposition is faster and more efficient under oxic condition has been questioned with experimental studies in peatlands and paddy fields, undergoing sequential dry-oxic and wet-anoxic conditions (Guntiñas et al., 2009; Fenner & Freeman, 2011). In boreal fen, subject to water-level fluctuations, carbon mineralization rates in oxic and anoxic conditions were only marginally different from each other (Kane et al., 2013). Further studies demonstrated that carbon mineralization rates in paddy soils exposed to weekly transitions from oxic to anoxic conditions were not affected by oxygen availability (Hanke et al., 2013); the authors also hypothesize that a scarce availability of alternative (to oxygen) electron acceptors during flooded periods could limit the potential CO_2 production under anoxic conditions. These findings underline the need for a better understanding of the effects of intermittent oxygen availability on carbon mineralization rates.

Hypoxic or anoxic events may be occasional, persistent, or intermittent, depending on a suite of physical and biological factors (Ford et al., 2002; Quiñones-Rivera et al., 2010). In the monimolimnion of meromictic lakes, anoxia may last for years, it being ended only by water overturn. In productive wetlands, high water temperatures and stagnation, which typically occur in summer, can lead to oxygen shortage due to elevated metabolic rates coupled with low gas solubility (Kemp et al., 2005; Maerki et al., 2009). Here, hypoxia or anoxia events may last from hours, depending on diel photosynthetic and respiration patterns, to days due to other forcing factors.

In heavily exploited river floodplains, riverine wetlands are affected by eutrophication which often stimulates the evolution of macrophyte communities from submerged toward surface-floating canopies (Ribaudo et al., 2011). In turn, the different macrophyte typologies are responsible for OM enrichment and changes in daily and seasonal oxygen budgets. At high temperatures, from late spring through summer under temperate conditions, OM availability causes the oxygen respiration to overwhelm the photosynthetic production with markedly negative daily oxygen budgets, associated with net CO_2 and methane effluxes from water to atmosphere (Bolpagni et al., 2007; Ribaudo et al., 2011). In temperate wetlands, anoxia may also establish in winter months, when photosynthesis is negligible and a thick ice cover hampers oxygen diffusion from the atmosphere to the water mass.

The persistent oxygen deficiency has rather predictable and generally well-documented effects on the benthic system, the most severe of which is the loss of benthic fauna, including subsurface or deep burrowers that provide gas and solute exchanges between sediment and water. As bioturbation is recognized to be responsible for enhanced microbial metabolism and OM degradation, the benthic macrofauna loss due to anoxia should hamper decomposition thus favoring carbon preservation (Kristensen, 2000). However, in such ecosystems where oxygen concentrations may widely fluctuate during the day, from super-saturation to anoxia, bacterial and faunal communities are less affected by oxygen shortage, to which they can adapt through physiological and/or behavioral responses (Rose & Crumpton, 1996).

In the floodplain, eutrophication processes are often stimulated by the large availability of nitrate from agriculture and livestock (Bartoli et al., 2012). However, when hypoxia or anoxia establishes, nitrate may also act as an electron acceptor alternative to oxygen, thus stimulating OM mineralization through nitrate reduction and denitrification. In freshwater ecosystem enriched by OM, anoxia usually results in elevated methane effluxes. Paradoxically, methane emission can be reduced by nitrate pollution. This is a key point as the number of aquatic ecosystems with concurrent nitrate pollution and oxygen shortage is increasing throughout the world (Smith et al., 1999; Diaz & Rosenberg, 2008).

Our study aims at analyzing the seasonal changes of organic carbon mineralization in a wetland system where anoxia frequently establishes and where nitrate can compensate for oxygen deficiency as an alternative electron acceptor. More specifically, the investigation had a twofold goal: (i) to compare carbon mineralization under oxic and anoxic conditions and (ii) to evaluate the effects of temperature and nitrate availability on benthic mineralization.

We hypothesized that in freshwater wetlands subjected to frequent oxygen fluctuations, rates of carbon mineralization under oxic and anoxic conditions should not significantly differ, because (1) the shift between the oxic and anoxic conditions is a rule here and not a catastrophic, infrequent event, and (2) nitrate availability may mitigate the oxygen shortage stimulating the anaerobic oxidizing microbial metabolism. We therefore consider that in those ecosystems where anoxia regularly establishes but with nitrate availability, the microbiota may vary its metabolism without appreciable changes in OM decomposition rates.

In order to reach these aims, we investigated carbon mineralization rates (O_2 consumption, CO_2 and CH_4 production) and sediment–water fluxes of inorganic nitrogen with intact core incubation, performed during diel transitions from oxic to anoxic conditions. Experiments were carried out seasonally, 1 year round, in a freshwater wetland in the Po river floodplain, Italy, where water temperature and nitrate undergo wide seasonal variations. Preliminary studies of denitrification were also performed at two different temperatures, which are expected to differentially affect microbial processes: <10°C in March and 24–25°C in July and September.

Materials and methods

Study site

The study was carried out in the Natural Reserve “Paludi del Busatello,” a wetland complex remaining after reclamation in the nineteenth century (11°05′59″E; 45°06′16″N). The wetland is composed of 14 ha ponds and narrow channels with a mean water depth of about 1 m, and 67 ha stands of common reed (*Phragmites australis*) and sedges (*Carex* spp.). The wetland is fed by drainage waters from the surrounding farmland; water levels are fairly constant over the year. The ponds and channels are colonized by dense meadows of floating leaved and submerged macrophytes whose biomass accumulates at the sediment surface, which is fluffy with OM > 30%. Under these conditions, anoxia frequently occurs, also in the water column, especially at night in the summer period due to water stagnation and temperatures >30°C (Fig. 1), while during winter anoxia could occur as a consequence of extended periods of ice cover. Like temperature and dissolved oxygen, also the amount of nitrate in the water follows a pronounced seasonal

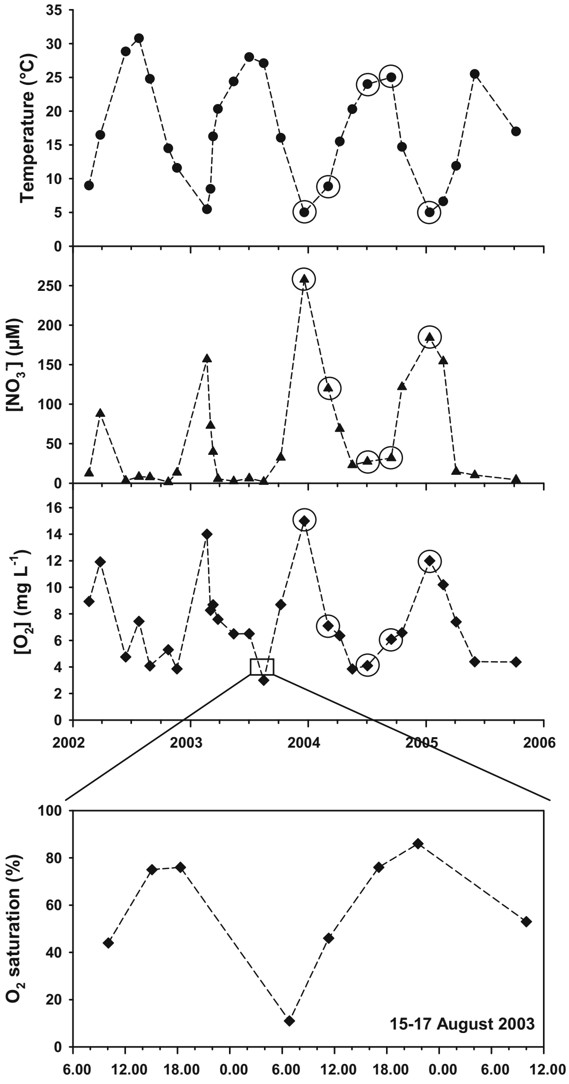


Fig. 1 Seasonal pattern of water temperature and nitrate (NO_3^-) and oxygen (O_2) concentrations at the sampling station of a freshwater wetland of the Po river floodplain, Italy; the values are referred to the period of February 2002–October 2005. An example of O_2 fluctuations at daily scale during the summer period is also shown. Circles indicate the five sampling dates for water and sediments

trend, with winter peaks and lower summer values (Fig. 1).

Water and sediment sampling

Water and sediment were collected on five occasions in December 2003, March, July, and September 2004, and January 2005 in the northern portion of the Reserve. The sampling station was located in a shallow pond (~1 m) with fluffy unvegetated sediments. On each sampling occasion, three cores (inner diameter 20 cm and height 40 cm) were collected by hand for flux measurement, while in March, July, and September 2004 six additional cores (inner diameter 8 cm and height 40 cm) were collected for denitrification experiments. After sampling, part of the sediment in the bottom of each core was cut off leaving a sediment level inside cores of 20 cm and an overlying water column of about 10 cm. Liners were sealed at the bottom with rubber bungs and stored with the top open submersed in a box containing cooled water of the sampling station. Within 3 h from sampling, cores were transferred to the laboratory and left overnight in a tank containing aerated water maintained at the temperature measured in situ (±1°C). Water mixing inside cores was ensured with small pumps (20 cm i.d. cores) or magnetic bars driven by an external motor (8 cm i.d. cores); the mixing devices were suspended above the sediment and regulated to avoid sediment resuspension. Approximately 200 l of site water were used for core maintenance during transport, pre-incubation, and incubation periods.

On each date, water temperature and dissolved oxygen concentration were measured in situ with a multiple probe (ISI Instruments, model 556, Twin Lakes, WI, USA). Water samples were collected by hand at 20 cm depth. Part of the water was filtered on site (GF/F, Whatman) for dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_2^- + NO_3^-$) determination; the analysis was performed in the laboratory with standard spectrophotometric methods (Koroleff, 1970; APHA, 1998).

Measures of sediment–water fluxes

The day after the sampling, water inside the incubation tank and inside the cores was replaced with fresh water from the sampling site. The dark incubation started just after when floating Plexiglass lids were positioned on the top of each core. At the beginning of the incubation, water samples (~100 ml) were collected in triplicate with a syringe from the tank and then, at different time intervals, from a valve on the top of each lid. Samples for dissolved oxygen, dissolved inorganic carbon (DIC), and methane were transferred into 12-ml glass tubes (Exetainers, Labco, Lampeter, UK). Oxygen was determined with the Winkler iodometric method (APHA, 1998), DIC with HCl titration (Anderson et al., 1986), and methane with gas chromatography (Fison GC 9000, FID detector). Samples for DIN were filtered (GF/F, Whatman), transferred into polycarbonate vials, and analyzed with standard spectrophotometric methods (Koroleff, 1970; APHA, 1998).

The total incubation time ranged between 22.5 and 58.5 h, depending on the water temperature, which allowed the calculation of fluxes when overlying water was oxic (at the beginning of the incubation) and when overlying water was anoxic (at the end of the incubation). Fluxes were determined according to the equation:

$$F_x = \alpha \cdot h \cdot 10,$$

where F_x (μmol m^{-2} h^{-1}) is the flux of the x species, α (μM h^{-1}) is the slope of the linear regression between concentration of the x species and incubation time, h (cm) is the height of the overlying water column, and 10 is a factor necessary to convert units. For a more detailed description of employed materials and pre-incubation and incubation techniques, see Dalsgaard et al. (2000).

Determination of denitrification rates

Denitrification rates were measured in 8 cm i.d. cores according to the isotope pairing technique (Nielsen, 1992). Triplicate incubations were performed for both oxic and anoxic conditions in March (8°C), July (24°C), and September (25°C) 2004.

At the beginning of the experiment, the water in the tank was lowered to below the core tops and a labeled nitrate ($^{15}NO_3^-$) solution (98 at.%, Sigma-Aldrich) was added to give a final concentration of ~30 μM. In order to calculate the $^{14}N/^{15}N$ ratio in the nitrate pool, the nitrate concentration was measured prior to the addition of the $^{15}NO_3^-$ solution and then 5 min after. Following the addition of $^{15}NO_3^-$, cores were pre-incubated from 15 min (summer) to 30 min (spring) before the start of the incubation to allow the tracer to diffuse to the sediment nitrate reduction zone (Nielsen, 1992). The incubation started thereafter when cores were closed using floating Plexiglass lids. At the end of the incubations, 10 ml of a $ZnCl_2$ solution (7 M) was added to the water phase of each core in order to prevent further denitrification, and dissolved N_2 pools in the water column and sediment pore water were homogenized by gently mixing the sediment with a metal bar. Subsamples of the final slurry were transferred to 12-ml gas-tight vials (Exetainer, Labco, High Wycombe, UK), fixed with 100 µl 7 M $ZnCl_2$, and stored at 4°C until analyzed. The $^{14}N^{15}N$ and $^{15}N^{15}N$ abundance in N_2 were analyzed by mass spectrometry at the National Environmental Research Agency, Silkeborg, Denmark. The rates of denitrification were calculated according to the equations and assumptions of Nielsen (1992).

Statistical analysis

Data were analyzed using generalized least square (GLS) models. GLS allows for heterogeneity of variance to be incorporated in the model and it is essentially a weighted linear regression. Condition, date, and their interaction were considered as fixed factors; heterogeneity of variance due to the factor date was accommodated with the varIdent function of the nlme package. A posteriori comparison of the means was performed using a Tukey HSD test.

All statistical analyses were performed using the statistical computing software R (R Core Team, 2014) with the packages “nlme” (Pinheiro et al., 2014) and “lsmeans” (Lenth, 2015).

Results

Temperature and oxygen and nitrate concentrations in the water column followed a seasonal trend (Fig. 1): DIN and oxygen peaked in winter and attained the lowest values from July to September. DIN was composed mainly of nitrate that attained very high concentrations in winter, while ammonium was dominant in summer.

Sediment oxygen demand (SOD) and DIC effluxes under oxic conditions differed significantly between sampling dates ($P < 0.01$) with peaks in July (SOD = −56.9 ± 5.0; DIC = 85.3 ± 7.7 mmol m^{-2} $days^{-1}$) and September (SOD = −57.0 ± 23.7; DIC = 110.3 ± 22.7 mmol m^{-2} $days^{-1}$) and the lowest in December (SOD = −13.1 ± 4.8; DIC = 18.4 ± 4.7 mmol m^{-2} $days^{-1}$) ($P < 0.05$, Fig. 2). The post hoc analysis revealed that SOD and DIC fluxes were similar in July and September and that September rates were significantly higher than those measured in December, January, and March (Tukey HSD test, $P < 0.05$). Respiratory quotient (RQ = DIC efflux/O_2 influx) ranged between 1.02 ± 0.11 (January) and 2.21 ± 1.01 (September).

The analysis of variance revealed that DIC effluxes differed significantly among sampling periods and incubation conditions ($P < 0.05$), with DIC effluxes under anoxia larger than under normoxia. However, the post hoc comparison of means did not produce significant differences within the sampling dates, likely due to large variability among replicates. Interestingly, DIC efflux tended to increase only slightly in July and September, while in colder months differences between mean oxic and anoxic rates varied from +91 to +147% (Fig. 2).

Net methane exchanges across the sediment–water interface differed significantly between incubation conditions (oxic versus anoxic), but differences depended upon sampling dates (significant date x condition interaction, $P < 0.01$). Efflux rates were negligible at low water temperature, while they increased significantly at higher temperatures in July and September (Fig. 3). In the shift from oxic to anoxic conditions, rates of net methane release nearly doubled in July (from 2.2 ± 0.0 to 5.6 ± 3.4 mmol m^{-2} $days^{-1}$) and September (from 0.5 ± 0.2 to 1.1 ± 0.5 mmol m^{-2} $days^{-1}$) (Fig. 3).

Nitrate fluxes were always negative both under oxic and anoxic conditions and were characterized by large variability (Fig. 4a). Differences depended upon both factors sampling date ($P < 0.01$) and incubation conditions ($P < 0.05$). The maximum nitrate uptake was measured in January (−8.8 ± 3.5 and −8.5 ± 3.3 mmol m^{-2} $days^{-1}$ for the oxic and anoxic conditions, respectively) when water column nitrate concentration peaked. The lowest uptake occurred in July in both oxic and anoxic conditions (−0.5 ± 1.4 and −0.6 ± 0.8 mmol m^{-2} $days^{-1}$, respectively), likely due to the lack of nitrate in the

Fig. 2 Dark sediment oxygen demand (O_2) and dissolved inorganic carbon (DIC) fluxes (mean ± SD; $n = 3$) measured in the oxic and anoxic phases of the incubation of sediments from a wetland of the Po river floodplain, Italy

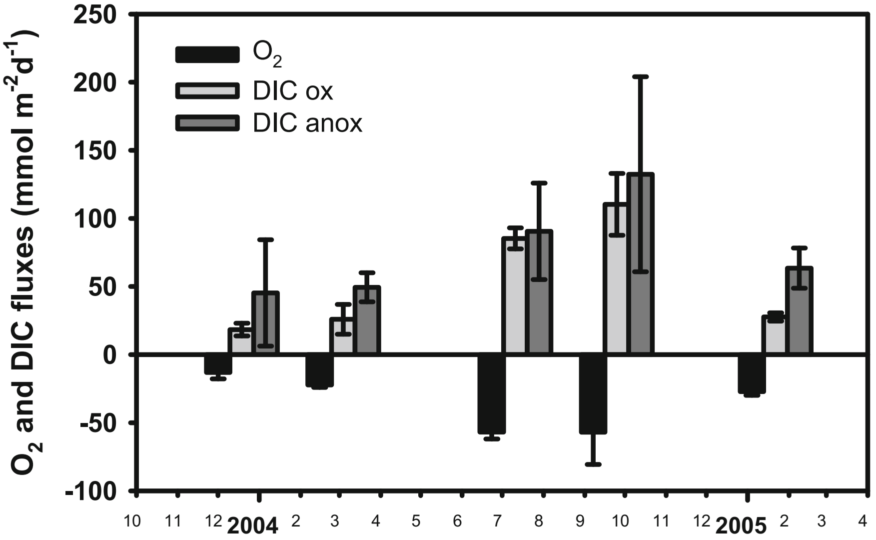
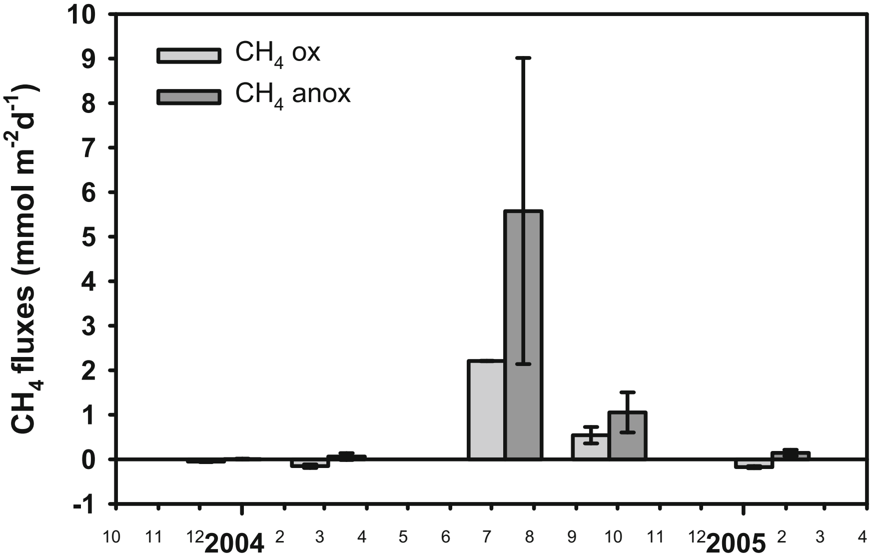


Fig. 3 Net methane (CH_4) fluxes (mean ± SD; $n = 3$) measured in the oxic and anoxic parts of the incubation of sediments from a wetland of the Po river floodplain, Italy

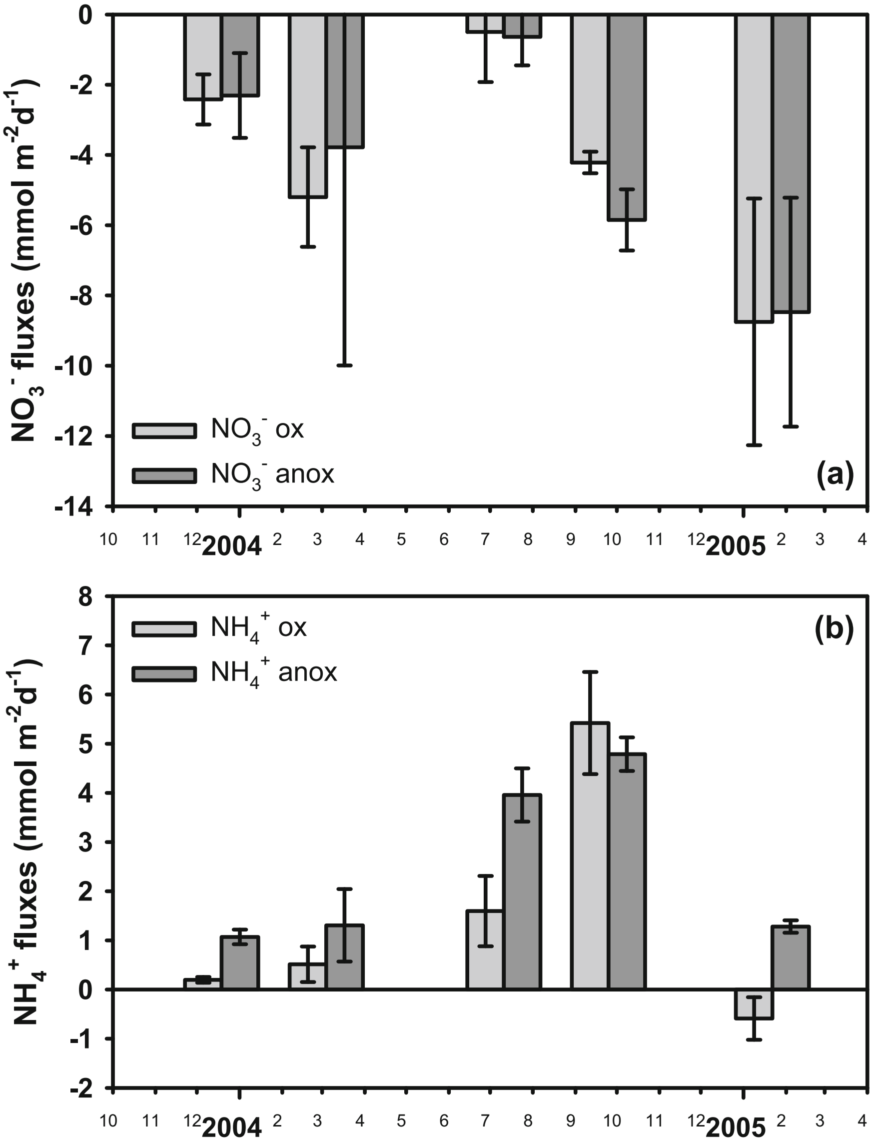


water column. The post hoc comparison of oxic versus anoxic incubations revealed significantly different nitrate uptake only in September (Tukey HSD test, $P < 0.01$), with higher consumption under anoxia.

Differences among ammonium fluxes depended upon the interaction of the two factors sampling date and incubation condition ($P < 0.01$). Except for the oxic incubation of January, ammonium was always released to the water column with rates ranging between 0.20 ± 0.06 (oxic phase, December) and 5.42 ± 1.04 mmol m^{-2} $days^{-1}$ (oxic phase, September) (Fig. 4b). Ammonium regeneration followed a seasonal trend with a late summer maximum under both incubation conditions. The post hoc analysis revealed that summer rates (July and September) were significantly higher than those measured in December, January, and March (Tukey HSD test, $P < 0.05$), and that the oxic-to-anoxic shift produced significantly different ammonium fluxes in January and July (Tukey HSD test, $P < 0.01$) (Fig. 4b).

Denitrification rates were constrained by the temperature in March, when dissolved nitrate was abundant in the water column, and by nitrate availability in summer (Table 1; Fig. 1). The differences between

Fig. 4 Net nitrate (NO_3^-) (**a**) and ammonium (NH_4^+) (**b**) fluxes (mean ± SD; $n = 3$) measured in the oxic and anoxic phases of the incubation of sediments from a wetland of the Po river floodplain, Italy



oxic and anoxic conditions were statistically significant only in September, when the high denitrification rates measured under oxic conditions more than halved (Table 1).

Discussion

Oxic–anoxic transitions in temperate floodplain wetlands

Relict wetlands, as our study case, consist of rapidly infilling, organic- and often nutrient-rich aquatic environments, undergoing rapid eutrophication. Most of them are isolated from main water bodies as rivers or large lakes and are therefore stagnant, bordered by heavily fertilized farmland, and prone to diffuse pollution (Bartoli et al., 2012). Our data highlight the wide range of physico-chemical conditions of the shallow water column, with large seasonal variation in water temperature and oxygen and nitrate availability (Fig. 1). Typically, maximum water temperatures of the dry period are coupled with minimum concentrations of the most energy-yielding electron acceptors, oxygen and nitrate, that peak in winter (Racchetti et al., 2011). Our results confirm the two working

Table 1 Denitrification rates (mmol m^{-2} $days^{-1}$) and associated carbon mineralization rates (mmol m^{-2} $days^{-1}$) in a wetland of the Po river floodplain (Italy) calculated using a stoichiometric relationship of 1.25 mol of DIC (dissolved inorganic carbon) produced every 1 mol of nitrate reduced

Sampling date	Denitrification		Carbon mineralization	
	Oxic	Anoxic	Oxic	Anoxic
March 2004	0.34 ± 0.05	0.36 ± 0.15	0.43 ± 0.06	0.45 ± 0.19
July 2004	1.14 ± 0.21	1.41 ± 0.20	1.43 ± 0.26	1.77 ± 0.25
September 2004	5.49 ± 1.26	1.94 ± 0.54	6.87 ± 1.57	2.43 ± 0.68

hypotheses as the short-term transition from oxic to anoxic condition that we induced did not result in an appreciable variation of the most synthetic proxy of benthic metabolism, which is DIC flux. They also confirmed how relevant, in the absence of oxygen, is the availability of nitrate. We speculate that low temperatures and elevated concentrations of nitrate are buffers that contrast the potentially negative impact of oxygen exhaustion, as for example the massive release of methane from sediments. On the contrary, we expect extreme reducing conditions of these systems in summer when oxygen depletion in organic sediment may make methanogenesis the main anaerobic pathway (Liikanen et al., 2002; Stadmark & Leonardson, 2007).

The build-up of anoxia in eutrophic shallow water bodies is frequently documented in summer due to low gas solubility, slow water renewal, and stagnation and high respiration rates. Changes in dissolved oxygen concentration up to 10 mg l^{-1}, associated with night anoxia, are for example reported in the summertime by Rose & Crumpton (1996) and were recorded also in wetlands and shallow waters in the studied area (Bolpagni et al., 2007). However, in our study we have simulated the build-up of anoxic conditions also in the winter period, when microbial activities are controlled by low temperatures and when the oxygen concentration in the water column is high. This may appear unlikely, but it may happen for example when thick ice cover develops on the water surface, preventing reaeration and oxygen exchange across the water–atmosphere interface (Barica & Mathias, 1979). In the wetland of this study, prolonged periods with ice cover, lasting for up to 2 months, were observed, together with massive death of fish communities caused by oxygen deficiency (data not shown).

For these reasons, we believe that transient or long-term anoxia may occur for different reasons all year round in these water bodies and that our simulations are not unrealistic. We acknowledge that our experimental setup allows only short-term effects of anoxia to be investigated as the long-term batch incubation of intact sediment cores results in solute accumulation in the water column and altered gradients, which can bias the estimation of benthic processes. Therefore, we tried to keep the whole incubation time to a minimum, which is 22–27 h in the warmer months and between 46 and 59 h in the cold periods.

Role of oxygen on sedimentary OM mineralization

While the effects of anoxia on benthic systems are generally well documented in lakes and coastal waters (Carignan & Lean, 1991; Liu et al., 1996; Viaroli & Christian, 2003; Middelburg & Levin, 2009), to our knowledge much less attention has been given to understand how the transition from oxic to anoxic conditions affects benthic metabolism in shallow freshwater ecosystems. In the studied wetland, oxygen penetration in the sediments varied between ~2 and 7 mm. Here, sediment porosity, density, and water and OM content were surprisingly homogeneous within the upper 10-cm layer, suggesting elevated rates of sedimentation together with frequent resuspension (Longhi et al., 2013). From these data, we assume that the most metabolically reactive OM was homogenously distributed in the upper 10-cm sediment horizon, where only a few uppermost millimeters were suitable for aerobic microbial processes. In these organic and fluffy sediments, electron acceptors as Mn^{4+} and Fe^{3+} are usually very low (Capone & Kiene, 1988). In turn, nitrate, which is the most energy-yielding electron acceptor after oxygen, is available only from autumn to early spring, due to surface runoff and slow microbial processes due to low temperatures. In summer, denitrification is hindered by nitrate exhaustion and by the inefficient nitrification due to oxygen shortage. Under these conditions, methanogenesis becomes quantitatively appreciable and likely the dominant microbial decomposition process (Lovley & Klug, 1982; Liu et al., 1996).

Nonetheless, our data suggest that the microbial metabolism of these systems cannot be predicted only from electron acceptor availability, but also by their time changes and interactions. The oxic–anoxic transition experiments indicated in fact that anoxia did not limit mineralization; on the contrary, anoxic conditions always resulted in enhanced DIC effluxes that could be interpreted as a higher net mineralization in the absence of oxygen. During the experiments, the change from oxic to anoxic conditions was sequential, therefore byproducts of aerobic metabolism may have fed anaerobic processes. In other words, under oxic conditions the refractory OM constituents can be transformed into more labile compounds that are more easily mineralized by the anaerobic microbial processes.

Usually, the efficiency of OM decomposition is regulated by its macromolecular composition (Bastviken et al., 2004). A common assumption is that the anaerobic mineralization is less efficient, especially when the organic substrates are refractory (Kristensen et al., 1995; Sun et al., 1997). Conversely, few studies have demonstrated that large DIC amounts can be produced under anoxia, as emerged from our work.

This typically occurs in anoxic organic soil with anaerobic bacterial communities. Here, oxygen penetration can stress the anaerobic microbial communities, hampering mineralization and enhancing carbon retention (Fenner & Freeman, 2011; Hanke et al., 2013). Anoxia can also result in a pH increase, allowing the desorption of dissolved organic carbon, previously retained in the solid phase, which becomes available for bacterial communities (Grybos et al., 2009).

Our results suggest that, more than oxygen availability, temperature was the main constraint of mineralization rates (Fig. 2) and, together with nitrate availability, of the dominant anaerobic path (methanogenesis, Fig. 3). At temperatures >20°C, the methane emission was greater at the lowest nitrate concentrations, under both oxic and anoxic conditions (Fig. 5a). At temperature >20°C, the methane emission was also inversely related to nitrate uptake (Fig. 5b).

Methane, nitrate, and ammonium fluxes in oxic and anoxic conditions

The transition from oxic to anoxic conditions stimulated methane effluxes, especially at high temperatures. Methane emission was directly related with water temperature and inversely with dissolved oxygen concentrations, since anoxia favors methane production and constrains the reoxidation of methane diffusing from sediments through the water column (Bastviken et al., 2004). At low temperatures, typical of winter and early spring (<10°C) in temperate

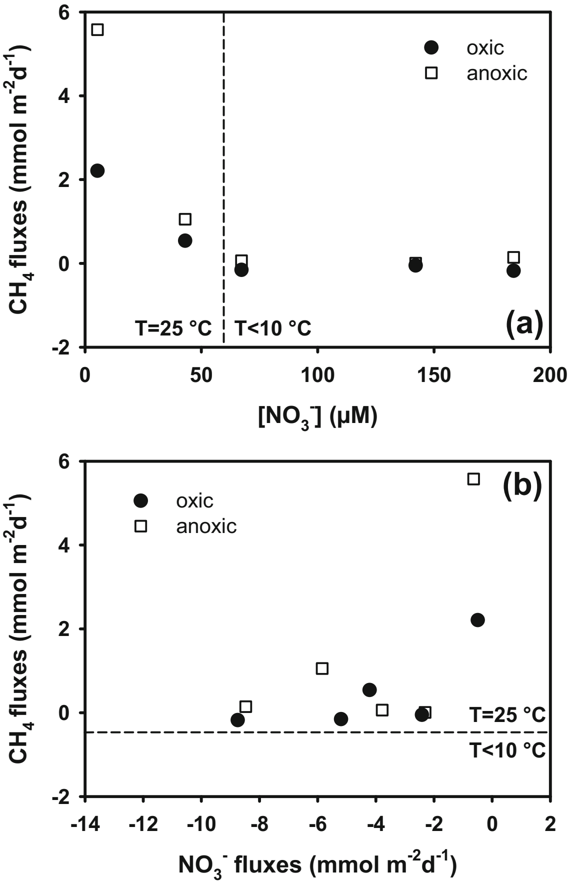


Fig. 5 Relationships between methane (CH_4) fluxes and nitrate (NO_3^-) concentrations (**a**) and between CH_4 fluxes and NO_3^- fluxes (**b**) in the sediments from a wetland of the Po river floodplain, Italy

wetlands, methane emission from water is very low and likely not related to the high nitrate concentrations, although nitrate can contribute to methane reoxidation (Smemo & Yavitt, 2011). However, the extent of the increase of methane fluxes when the system becomes anoxic seems related to both nitrate concentrations and fluxes in the water column (Fig. 5a, b). These results suggest that when oxygen is not available, nitrate can control net methane emission, supporting bacterial reoxidation in the proximity of the sediment–water interface (Smemo & Yavitt, 2011). Paradoxically, the increase of nitrate concentrations due to diffuse pollution may result in a lesser methane release due to coupled nitrate reduction and methane oxidation, with implications for both water quality and greenhouse gas production.

Nitrate fluxes were always directed toward the sediment, evidencing a net nitrate consumption by the benthic system. The nitrate removal was in part accounted for by denitrification, especially at high temperatures (July and September), while at $T < 10°C$ only ~20% nitrate was processed by denitrification, suggesting the relevance of other assimilative or dissimilative processes, the latter leading to ammonium. As nitrate is the dominant inorganic N fraction, a large bacterial uptake of nitrate is not surprising in this study area, due to the general refractory constitution of the OM pool, with an ecological stoichiometry of C and N unbalanced toward C excess.

We found slight differences between denitrification in oxic and anoxic cores, with the only exception being the September sampling. Our interpretation for the results of September deals with the relative importance of denitrification coupled to nitrification in this sampling period, when the co-occurrence of ammonium and oxygen within sediment may stimulate elevated nitrification rates in the oxic part of the incubation. Alternatively, the build-up of anoxia in the late summer may favor the vertical migration of reduced chemical forms that may inhibit denitrification. This is a reasonable explanation for the shift between denitrification and the dissimilative nitrate reduction to ammonium (DNRA) in organic-enriched sediment under low nitrate availability (Nizzoli et al., 2010). However, the higher nitrate consumption measured in September under anoxic conditions was not coupled to an equivalent higher production of ammonium. These incoherent results could be due to a methodological limit of our approach. September was in fact the period with the highest ammonium regeneration in the initial oxic part of the incubation and it is likely that the accumulation of ammonium in the water phase may have altered the solute gradient between pore and bottom water (Fig. 4). Future studies should address the relevance of DNRA in this study area; in eutrophic anoxic lakes, the shift between denitrification and DNRA was demonstrated, but overall the rates of C oxidation supported by these processes were low (Nizzoli et al., 2010).

Our data also suggest that the contribution of denitrification to C mineralization was generally low under both oxic and anoxic conditions, with fractions of 1–2, 2–3, and 2–7% of the total C oxidation in March, July, and September, respectively (Table 1 and Fig. 2). Furthermore, denitrification seemed to be a transient process, depending on nitrate availability in summer and temperature constraints in winter (Table 1).

The transition from oxic to anoxic conditions generally resulted in enhanced effluxes of ammonium, with the only exception being September 2004. Higher ammonium fluxes may be explained in terms of decreased nitrification, increased ammonification of dissolved OM released under oxic conditions, or dissimilative reduction of nitrate to ammonium. Regardless of the underlying process, this result is in part unexpected considering the OM quality at the study site (Bastviken et al., 2004). Occasional anoxia results at this site in a pulse release of ammonium to the water column. Overall, ammonium and DIC fluxes

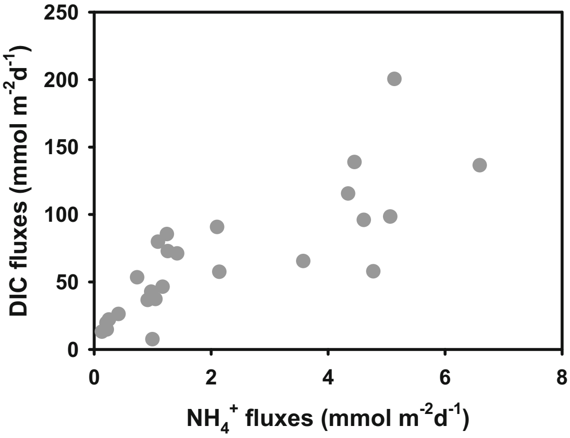


Fig. 6 Relationships between ammonium (NH_4^+) fluxes and dissolved inorganic carbon (DIC) fluxes in the sediments from a wetland of the Po river floodplain, Italy

were significantly correlated (Fig. 6); the slope of the regression between inorganic carbon and ammonium ($18.9, r = 0.80, P < 0.01$) was very similar to the C:N ratio of the macrophytic vegetation, previously reported (Longhi et al., 2008). The shift from oxic to anoxic conditions does not seem to deeply impact benthic N cycling at the study site: sediments resulted in net inorganic nitrogen sinks, with nitrate uptake more than compensating ammonium efflux. The only exception to this is the summer, when ammonium recycling dominates and it is strongly stimulated by anoxia. The summer period remains the most critical period for the effects of anoxia, as also evident for methane fluxes.

Concluding remarks

In eutrophic wetlands in the flood plain, frequent oxic–anoxic transitions can affect OM mineralization making it less dependent on oxygen availability. Multiple factors can affect decomposition processes: among others, temperature is a clear driver, but above certain temperatures the availability or the absence of nitrates can regulate methane emission. SOD and methane and ammonium fluxes are indeed directly related to water temperature. However, it is interesting to highlight that at high temperatures methane effluxes peak when nitrate concentration, nitrate uptake, and denitrification are low. Therefore, nitrate seems to act as a switch regulating the metabolism, when it becomes anaerobic and dominated by reduction pathways.

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