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ORIGINAL PAPER

Contrasting factors controlling microbial respiratory activity in the sediment of two adjacent Mediterranean wetlands

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Abstract Electron transport system (ETS) activity of sediments as an indication of microbial metabolic activity was examined in two adjacent Mediterranean wetlands (southern Spain). We determined the spatio-temporal variation in ETS, and we explored the potential biological [organic matter (OM), chlorophyll a (Chl a), aerobic and anaerobic bacteria] drivers of sediment ETS activity. ETS activity was notably higher in the eutrophic Lake Nueva (34.91 μl O_2 $g^{-1}D.W.h^{-1}$) than in the hypertrophic Lake Honda (24.99 μl $O_2 g^{-1}D.W.h^{-1}$). Strong spatial differences were observed in ETS in both study sites. Highest ETS values were achieved at the surface sediment at the deepest sampling station in each lake and a notable reduction in ETS with sediment depth was observed. By using linear regression and multiple regression analysis, OM was identified as the best predictor of ETS in Lake Honda while Chl a was the best predictor in Lake Nueva. The strong influence of OM supply on ETS activity in sediment from Lake Honda was the consequence of the labile nature of sedimentary OM, while a more refractory OM (with a higher contribution of vascular plants) comprised most of the sedimentary OM from Lake Nueva. By contrast, a large contribution of phytobenthos (supported by a higher lake water transparency) to ETS has been recognized in sediments from Lake Nueva. In summary, the results of this study revealed that the relative importance of planktonic primary producers (phytoplankton), benthic algae and vascular plants in the study sites could explain the differences observed in the intensity of sediment ETS as well as in their drivers.

Keywords Electron transport system · Benthic metabolism · Sediments · Wetlands

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Introduction

The rate of in situ processes, photosynthesis and respiration, is essential in the construction of dynamic models of the carbon (C) cycle in aquatic ecosystems (Rai 1988). On a global scale, rates of material processing (e.g., C, nutrients) by aquatic ecosystems are likely to be at least twice as important as had been previously supposed (Downing et al. 2006). Even more, since the numerical and areal cover of small water bodies is much greater than was previously considered, processes that are most active in small lakes and ponds may assume global significance (Downing et al. 2006). In conjunction with photosynthesis, respiration determines net production, growth potential, and production efficiency (Margalef 1974). Primary production by phytoplankton, macrophytes, and benthic algae forms the autochthonous basis of the food web, while terrestrial input of particulate and dissolved organic carbon is a potential allochthonous basis of the food web (Cole et al. 2000; Simčič and Germ 2009). In shallow aquatic environments, the sediment bed is the most important site for respiration of organic matter to which it strongly contributes the benthic (meio- and macro-) fauna (Relexans

1996a). Accordingly, the measurement of sediment community respiration is essential for knowing the turnover of organic matter in sediments (Andersen and Helder 1987).

Respiration in aquatic systems is usually determined by direct measurement of oxygen uptake (Andersen and Helder 1987; Simčič and Mori 2007). Since oxygen consumption measurement is time-consuming and in the case of low metabolic activity of organism it also has low sensibility, other methods have been developed to evaluate microbial respiration and cell viability (Simčič and Mori 2007). As most oxidation of organic matter occurs in organisms having respiratory chains, an overall estimate of metabolism (aerobic plus anaerobic) can be obtained by measuring the activity through the respiratory chain (Relexans 1996a). The method was first proposed by Packard (1971) for measuring the electron transport system (ETS) activity in marine phytoplankton. Since 1970s, the method has been widely used for measuring ETS in marine plankton (Bamstedt 1980; Kenner and Ahmed 1975; Ramírez et al. 2006; Savenkoff et al. 1996; Vosjan and Olanczuk-Neyman 1991), in freshwater plankton (Borgmann 1978; del Giorgio 1992; James 1987; Jones and Simon 1979; Simčič and Germ 2009), in benthic organism (Cammen et al. 1990), in leaf litter (Fleituch and Leichtfried 2007), in marine sediments (Christensen 1983; Christensen and Packard 1977; Olanczuk-Neyman and Vosjan 1977; Relexans 1996a, b; Vosjan and Olanczuk-Neyman 1977), and in freshwater sediments (Muri and Simčič 2004; Simčič 2005; Simčič and Brancelj 2002; Simčič and Germ 2009; Simčič and Mori 2007; G.-Tóth 1992; G.-Tóth et al. 1994; Trevors 1984). Finally, production in ETS assays has been found to be closely correlated to oxygen consumption (del Giorgio 1992).

Previous studies have shown that metabolic activity in the sediment depends on a variety of single biological and chemical factors (see among others, Simčič and Brancelj 2002). However, up to date, there is not a comprehensive study for elucidating the main factors responsible of sediment ETS activity. Therefore, in the present study, we determined the spatio-temporal variation in ETS in two adjacent shallow lakes. In addition, we explored the potential biological (organic matter availability, chlorophyll a, aerobic and anaerobic bacteria) drivers of sediment ETS activity.

Material and methods

Study site

Albufera de Adra, composed of two small shallow coastal lakes, Honda and Nueva, is one of the most important wetlands in southeastern Spain (de Vicente et al. 2003, 2010; Fig. 1). Both systems can be considered as polymictic due to their shallowness and the intensity and frequency of the prevailing winds. These ecosystems had a pH >8 and a high alkalinity (3.17–6.21 meq l^{-1} and 1.46–3.14 meq l^{-1} in Lake Honda and Lake Nueva, respectively) for most part of the year (de Vicente et al. 2003). Conductivity values ranged from 1.42 to 7.31 mS cm^{-1} in Lake Honda and from 3.94 to 7.39 mS cm^{-1} in Lake Nueva (Moreno-Ostos et al. 2007). Despite the ecological significance of the wetland, high internal P loadings to both study lakes and external just for the case of Lake Honda (1.73 gP m^{-2} $year^{-1}$) have promoted eutrophication in both lakes, especially in Lake Honda (Table 1). Both lakes can be considered as pelagic-oriented systems, although in Lake Nueva a simple macrophyte-dominated benthos community developed during certain times of the year (de Vicente et al. 2003, 2010). The annual average water transparency is greater in Lake Nueva where values for the photon flux (PAR) at the deepest part of the lake reflected that euphotic zone extended to the bottom and allowed the development of patches of submerged macrophytes (de Vicente et al. 2003). In Lake Honda, the transparency was much less (Table 1) and the mixing depth was, for most of the year, greater than the euphotic zone, hence restricting the primary production in the system (Cruz-Pizarro et al. 2002). Although both study lakes show a notable temporal variability, Lake Honda is characterized by large long-term, seasonal, and diel fluctuations in the water quality (de Vicente et al. 2006a). Instability and extreme fluctuations in

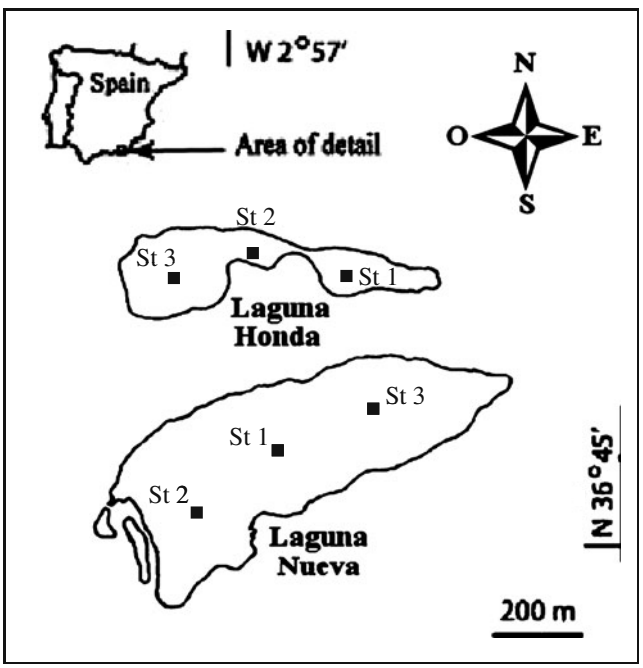

Fig. 1 Geographical location of study sites. *Squares* represent sampling stations for the study (modified from de Vicente et al. 2010)

Table 1 Main morphometric features of the investigated lakes (modified from de Vicente et al. 2003)

	Lake Honda	Lake Nueva
Lake area ($10^3 m^2$)	94	271
Volume ($10^3 m^3$)	118	627
Mean depth (m)	1.26	2.32
Maximal depth (m)	3.19	3.80
Residence time (yr)[1]	0.17	2.95
Watershed area ($10^6 m^2$)	13.7	0.5
External Areal loading (Lp) (g m^{-2} yr^{-1})[a]	1.73	0.03
Total P (μg l^{-1}) (annual mean)[a]	312	76
Chlorophyll a (μg l^{-1}) (annual mean)[a]	153	54
Chlorophyll a (μg l^{-1}) (annual peak)[a]	410	143
Secchi Depth (m) (annual mean)[a]	0.37	1.30

[a] Values corresponding for the period March 2000–February 2001

Lake Honda water quality are to a large extent regulated by rapid changes in the internal supply rates of nutrients, as a result of intense biological, physical, and chemical mechanisms at the sediment–water interface (de Vicente et al. 2006b).

Sediment sampling

Sediment samples were collected during 2002 on a seasonal basis, at three different sites in Lake Honda and Lake Nueva (Fig. 1), using an Ekman bottom grab sampler. Each sample was sliced, in situ, into three different layers: 0–5, 5–10, and 10–15 cm.

Sediment ETS activity

ETS activity was measured by using the method of Broberg (1985) with slight modifications. This method determines the capacity of the respiratory chain to transfer electrons from the physiological substrates (NADH, NADPH, succinate) to oxygen (Packard 1971, 1985). It is mainly based on the biological reduction of the tetrazolium salts to their respective tetrazolium formazan by sediment microorganisms. The formazan produced can then be used as a measure of the ETS in sediment. For optimizing the method, a set of preliminary experiments were performed in order to determine the amount of optimized wet sediment and also to establish the saturation concentration of substrates to achieve V_{max} of the INT (2-(p-iodophenil)-3-(p-nitrophenol)-5 phenil tetrazolium chloride) reduction.

Based on the results obtained in the preliminary experiments, for measuring ETS in the sediment of the study lakes, 2–3 g of wet sediment was mixed with 10 ml of homogenate buffer ($MgSO_4 \cdot 7H_2O$, PVP, Triton, and EDTA) and sonicated in an ice bath for 4 min (0°C). The mixture was clarified by centrifuging at 10,000 rpm for 10 min (0°C). Then, we mixed 0.5 ml of the supernatant with 1 ml of substrate solution (NADH, NADPH, and Na-succinate), 0.5 ml of INT, and 0.5 ml of the homogenate buffer. The mixture was incubated at the same temperature measured in the field (15–28°C) and for 20–30 min, depending on the temperature. Immediately after time incubation, the addition of Quench (phosphoric acid and formaldehyde in 1:1 proportion) was carried out in order to stop the reaction. Absorbance of the sample at 490 nm was read with a spectrophotometer. In calculation ETS, the molar adsorption coefficient of INT-formazan of 1.42 (Kenner and Ahmed 1975) was used. All ETS values were determined within 24 h of field sampling. Samples were corrected by considering two different blanks (Relexans 1996a). First, turbidity blanks were prepared adding homogenate buffer and Quench solution to an aliquot of the supernatant containing the sample. Considering that reduction of INT to formazan can occur in two ways: biological (enzymatic) and chemical (Relexans 1996a), a second blank (a chemical blank) was prepared by mixing INT and substrate solution, at the same concentration as in the samples, with homogenate buffer instead of sediment extract. Finally, the chemical reduction was subtracted from total reduction to obtain the enzymatic production of formazan.

Sediment characterization

Fresh sediments were also analyzed for organic matter (OM) content, chlorophyll a (Chl a), and aerobic and anaerobic bacteria. OM concentration was estimated as loss on ignition by weighting before and after combustion of dried sediment at 520°C for 3 h, which is a procedure minimizing carbonate dissociation (Dean 1974). Chl a was extracted in acetone (4°C, 24 h), and the absorbencies at 665 and 750 nm were measured in a spectrophotometer (Lorenzen 1967). Chl a was measured at the three mentioned sediment layers at station 1 while only the top sediment was analyzed at station 2 and 3 in each study site. Colony formation units (CFU) of anaerobic and aerobic

bacteria were quantified by counting the colony number on the Petri plates incubated under oxic and anoxic conditions.

Statistical analysis

All analyses were carried out in triplicates. Statistical analyses were performed using Statistica 7.0 Software (StatSoft Inc 1997). For *t* tests, unless otherwise stated, the significance level was set at $p<0.05$. Regression analyses were performed to assess the potential drivers of ETS. Data were log transformed to comply with the assumptions of regression analyses.

Results

Method setup

Results of preliminary experiments for measuring ETS revealed the need for considering the sample size as a key factor for optimizing the method (Fig. 2a). In fact, highest ETS values were measured when wet sediment was in the range of 2–3 g for both study sites. However, a drastic reduction in ETS was observed for higher sediment mass. Similarly, Broberg (1985) found that an increase in sediment mass caused an ineffective cell disruption. Another important parameter for optimizing the ETS method is the amount of saturating substrate solution (Fig. 2b). Our results evidenced that ETS increased when increasing the volume of substrate solution from 0.5 to 1 ml but no any further increase was observed for volumes higher than 1 ml. In view of these results, ETS was measured in the sediment of Lake Honda and Nueva considering 2–3 g of wet sediment and the addition of 1 ml of substrate solution.

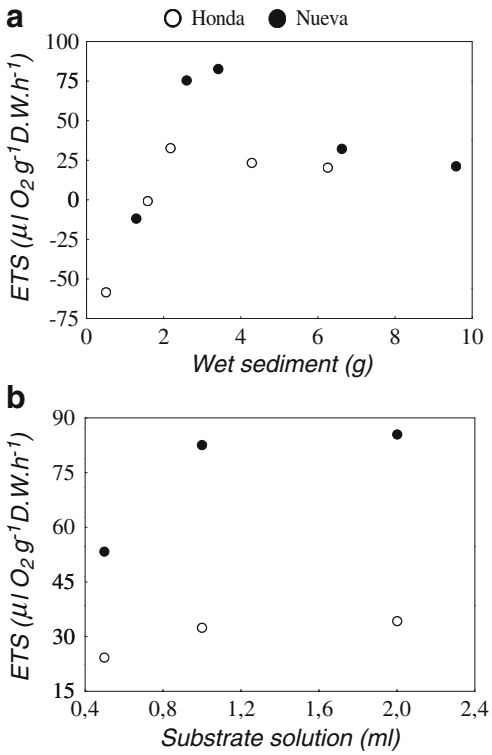

Fig. 2 Sediment ETS as a function of the amount of sediment (**a**) and of the volume of substrate solution (**b**)

ETS in lake sediments and potential drivers

ETS showed an average value of 24.99 µl $O_2 g^{-1} D.W.h^{-1}$ in Lake Honda and 34.91 µl $O_2 g^{-1} D.W.h^{-1}$ in Lake Nueva (Table 2). Seasonal variation of ETS was especially important for the case of Lake Nueva, reflected by the extremely high values of the coefficient of variation (CV >40 % in all cases). A drastic increase in ETS occurred in autumn 2002 when values increased up to twofold at the surface sediment at stations 1 and 2 and up to fivefold at station 3. Strong spatial differences were observed in ETS in Lake Honda and Nueva. Horizontal heterogeneity was similar in both study lakes, as CV of ETS in the surface sediment was 29% and 32% in Lake Honda and Nueva, respectively. Highest ETS values were achieved at the surface sediment at the deepest sampling station in each lake (station 1 in Lake Honda=50.1 µl $O_2 g^{-1} D.W.h^{-1}$and station 3 in Lake Nueva=81.9 µl $O_2 g^{-1} D.W.h^{-1}$). A notable reduction in ETS with sediment depth was observed in both study lakes. Hence, ETS was significantly higher at 0–5 cm than at 10–15 cm at the three sampling stations of Lake Honda and Nueva, except for station 3 of Lake Nueva.

OM content was significantly higher ($p<0.001$) in sediments from Lake Nueva (13.42%) than in Lake Honda (6.52%; Table 2). Significantly higher OM concentrations were measured in the upper (0–5 cm) than in the lower sediments (10–15 cm) in all sampling stations, and in both study lakes, except for the deepest point in Lake Honda (station 1), were an increasing tendency in OM content with depth was observed. Horizontal heterogeneity was especially relevant in Lake Honda, where OM content was close to twofold higher at the deepest station (station 1) than at the shallower stations (station 2 and 3).

Average Chl a concentration was 39.3 µg Chl a g^{-1} D.W. in Lake Honda and 54.9 µg Chl a g^{-1} D.W. in Lake Nueva (Table 2). While a notable reduction in Chl a concentration with depth was observed in Lake Nueva, it does not exist a clear pattern in Lake Honda. Highest Chl a concentration were recorded at surface sediment in the deepest stations of both lakes.

Aerobic and anaerobic bacteria abundances were significantly higher ($p<0.001$) in Lake Honda than in Lake Nueva (Table 2). Aerobic bacteria abundance was 7.19 and

Table 2 Mean (± SD) values of ETS (μl $O_2 g^{-1}$D.W.h^{-1}), OM (%), Chl a (μg g^{-1}D.W.), aerobic and anaerobic bacteria (log CFU g^{-1}D.W.) in the sediment of study sites

		Depth (cm)	ETS	OM	Chl a	Aerobic bacteria	Anaerobic bacteria
Lake Honda	Station 1	0–5	50.07 (±15.69)	8.5 (±1.9)	41.06 (±8.84)	7.41 (±0.18)	6.57 (±0.50)
		5–10	34.59 (±12.09)	9.7 (±0.6)	44.63 (±19.07)	7.21 (±0.52)	6.54 (±0.46)
		10–15	18.94 (±8.58)	11.7 (±1.0)	47.38 (±20.71)	7.11 (±0.17)	6.41 (±0.51)
	Station 2	0–5	28.01 (±9.10)	5.7 (±0.8)	24.65 (±7.44)	7.24 (±0.10)	6.40 (±0.46)
		5–10	18.00 (±9.29)	4.9 (±0.6)		7.07 (±0.17)	6.14 (±0.24)
		10–15	10.53 (±5.66)	4.3 (±0.9)		6.81 (±0.11)	6.08 (±0.20)
	Station 3	0–5	35.94 (±16.47)	5.5 (±0.5)	38.59 (±9.66)	7.22 (±0.13)	6.31 (±0.46)
		5–10	15.83 (±8.44)	4.1 (±0.5)		7.19 (±0.16)	6.25 (±0.02)
		10–15	10.96 (±5.32)	4.0 (±0.6)		7.02 (±0.08)	6.00 (±0.42)
Lake Nueva	Station 1	0–5	63.63 (±48.58)	16.6 (±1.4)	68.54 (±29.84)	7.0 (±0.25)	6.14 (±0.42)
		5–10	34.02 (±30.22)	14.0 (±3.0)	40.25 (±27.44)	6.69 (±0.50)	5.91 (±0.49)
		10–15	11.28 (±9.37)	10.0 (±2.9)	16.93 (±7.56)	6.43 (±0.33)	5.53 (±0.34)
	Station 2	0–5	42.16 (±21.35)	14.3 (±0.8)	37.22 (±9.47)	6.85 (±0.39)	6.14 (±0.36)
		5–10	25.73 (±12.43)	13.2 (±1.4)		6.53 (±0.33)	5.92 (±0.40)
		10–15	13.41 (±8.97)	10.4 (±1.8)		6.36 (±0.29)	5.67 (±0.24)
	Station 3	0–5	81.87 (±80.51)	18.2 (±1.3)	111.78 (±23.21)	6.94 (±0.26)	6.32 (±0.31)
		5–10	34.19 (±31.34)	15.0 (±4.0)		6.65 (±0.80)	6.07 (±0.25)
		10–15	11.07 (±17.60)	9.4 (±3.9)		6.22 (±0.43)	5.21 (±0.29)

6.69 log CFU g^{-1}D.W. in Lake Honda and Nueva, respectively, and anaerobic bacteria abundance was 6.36 log CFU g^{-1}D.W. in Lake Honda and 5.94 log CFU g^{-1}D.W. in Lake Nueva. In all of the three sampling stations of the study lakes, there exists a clear decreasing tendency with depth in both aerobic and anaerobic bacteria.

Considering ETS/OM ratio as a useful indicator of organic carbon quality, the average value was calculated in the study sites (Table 3). ETS/OM ratio was significantly higher ($p<0.001$) in Lake Honda than in Lake Nueva (Table 3). Similarly to ETS and OM content, there exists a clear decreasing pattern with depth.

Table 3 Mean values for the ETS/OM ratio

		Lake Honda		Lake Nueva	
	Depth (cm)	Average	SD	Average	SD
Station 1	0–5	5.95	1.86	3.82	2.73
	5–10	3.54	0.98	2.28	1.64
	10–15	1.61	0.64	1.25	1.04
Station 2	0–5	4.89	1.18	2.91	1.34
	5–10	3.19	1.58	2.07	0.97
	10–15	2.36	0.79	1.23	0.60
Station 3	0–5	6.54	2.52	4.36	3.75
	5–10	3.78	1.54	2.00	1.43
	10–15	2.83	1.26	0.96	0.98

To assess possible drivers of sediment ETS, we analyzed the relationships between ETS and different biological variables (OM, Chl a, aerobic and anaerobic bacteria) using linear regression (Fig. 3; Table 4). ETS and OM, Chl a, and aerobic and anaerobic bacteria were significant and positively related in Lake Nueva ($p<0.001$). However, ETS was only significantly related to OM content and aerobic bacteria in Lake Honda ($p<0.001$). To explore the relative contribution of each significant variable in determining ETS, we calculated their corresponding partial coefficients from a multiple regression analysis with OM and aerobic bacteria as the independent variables in Lake Honda and with OM, Chl a, and aerobic and anaerobic bacteria as the independent variables in Lake Nueva. The results indicated that OM was the best predictor of ETS in Lake Honda while Chl a was the best predictor in Lake Nueva.

Discussion

Inland waters have not been adequately considered in the context of the global C cycle, although currently these systems may sequester significant terrestrial organic C in sediments (Cole et al. 2007). Small and medium size lakes, because of their larger numbers and faster sediment accumulation rates, store even more C (Mulholland and Elwood 1982). However, the final role of small lakes as C sink or source depends on the net balance between primary production and respiration. As sediments in shallow lakes

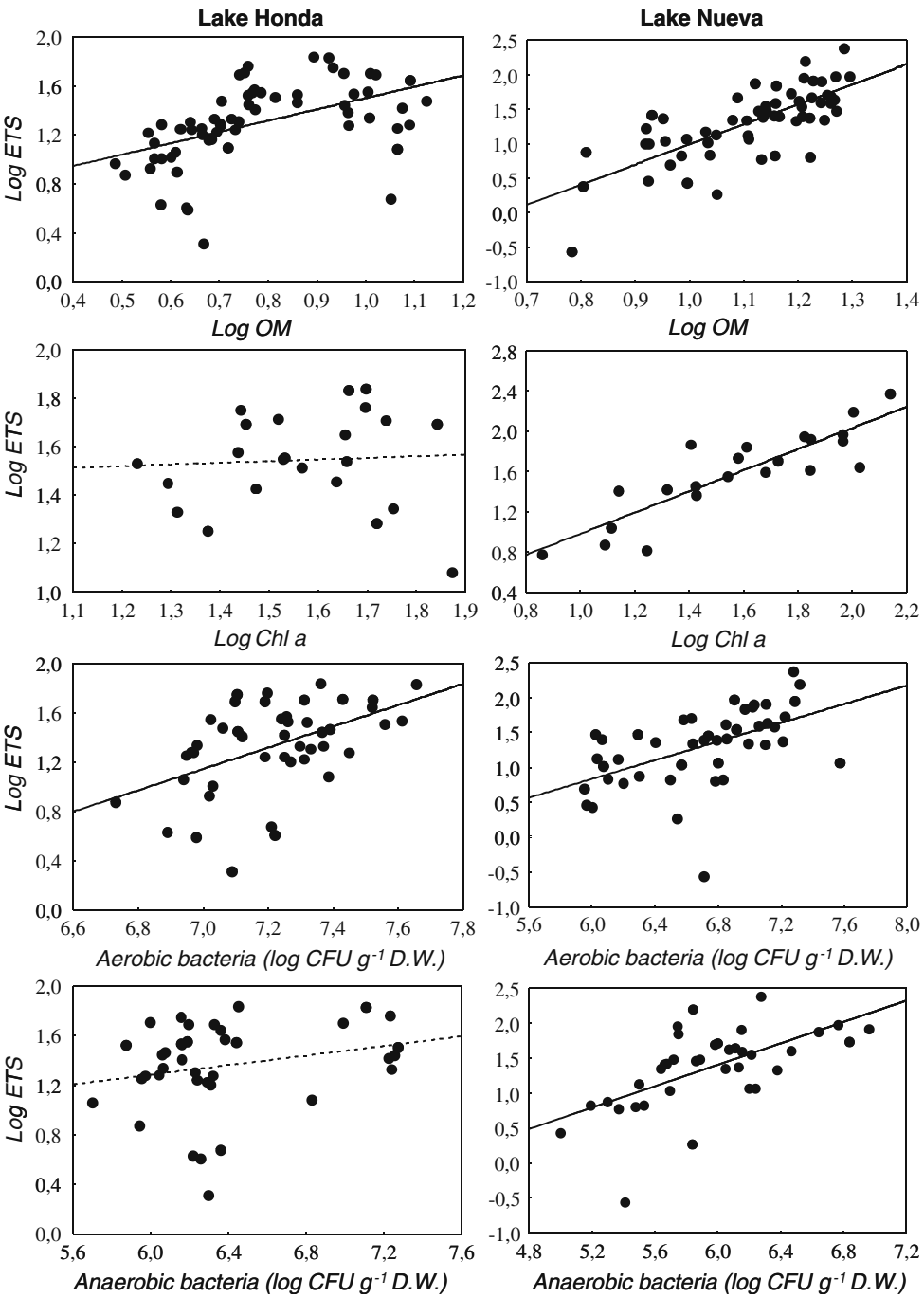

Fig. 3 *Scatter plots* between ETS and OM, Chl a, and aerobic and anaerobic bacteria in the sediment of Lake Honda and Lake Nueva. *Solid regression lines* are shown for significant relationships while *dashed lines* are shown for nonsignificant relationship

represent active sites for organic matter mineralization, an essential condition is to evaluate the potential for organic C mineralization of lake sediments as well as to identify the driving factors.

ETS in the eutrophic Lake Nueva was in the range of those values previously reported in the literature for freshwater sediments (i.e., Broberg 1985; Muri and Simčič 2004) while in the hypertrophic Lake Honda, ETS values were much lower (Table 5). Contrarily to our results, previous researchers have noted higher values of ETS in lakes with higher trophic level (Simčič and Brancelj 2002; G.-Tóth 1992; G.-Tóth et al. 1994), hence reflecting the complexity of factors involved in benthic metabolism.

There exists a clear decreasing tendency in ETS with depth. This pattern has also been observed in other studies (Andersen and Helder 1987; Broberg 1985; Relexans 1996b; Simčič and Brancelj 2002; G.-Tóth 1992). Several hypotheses for the sharp reduction in ETS with depth have been suggested. First, sediment stabilization and consolidation as well as the termination of aerobic metabolism may contribute to the reduction in ETS (Broberg 1985; Songster-Alpin and Klotz 1995). Second, as bacterial

Table 4 Results of the regression analyses performed between ETS and different variables

Dep Var	Indep Var	Lakes	Equations	r^2	p level	n
ETS	OM	Honda	$y = 0.93\,x + 0.58$	0.25	<0.001	62
		Nueva	$y = 2.90\,x - 1.92$	0.54	<0.001	62
	Chl *a*	Honda	$y = 0.07\,x + 1.44$	0.00	ns	23
		Nueva	$y = 1.05\,x - 0.07$	0.76	<0.001	23
	Aerobic bacteria	Honda	$y = 0.86\,x - 4.89$	0.24	<0.001	46
		Nueva	$y = 0.67\,x - 3.19$	0.28	<0.001	46
	Anaerobic bacteria	Honda	$y = 0.19\,x + 0.12$	0.06	ns	38
		Nueva	$y = 0.77\,x - 3.2$	0.37	<0.001	38

All variables were $\log_{10}$ transformed

numbers (Hayes and Anthony 1959) and benthic algae biomass (Simčič and Brancelj 2002) decline with depth, ETS activity is likely to decay. However, sediment from Lake Honda and Nueva can be considered biologically active up to 10–15 cm. Similarly, G.-Tóth (1992) noted that sediment was enzymatically active up to 30–35 cm in the hypertrophic Keszthely Bay of Lake Balaton (Hungary). As Simčič and Brancelj (2002) pointed out, the extension of ETS with depth generally depends on the lake trophic state. Accordingly, while intensity of ETS decline slowly in sediments from eutrophic systems, Relexans (1996b) observed that, in marine sediments from oligotrophic areas, ETS becomes undetectable deeper than 10 to 15 cm. Apart from the vertical gradient, study sites exhibited a notable horizontal heterogeneity in ETS with highest values at the deepest sampling stations in each lake, where higher sedimentation rates of OM are likely to occur. Similarly, Löfgren and Boström (1989) and G.-Tóth (1992) have detected strong spatial differences in ETS in lake sediments.

There is ample evidence that potential or actual metabolic activity in the sediment, or both, depends on microbial abundance (Jones and Simon 1979), depth of the water column (Christensen 1983; Christensen and Packard 1977; Relexans 1996a), availability of organic matter for organisms (Relexans 1996b; Trevors 1984), source of food (Broberg 1985; Zimmerman 1975), and zoobenthos abundance (Simčič 2005; Simčič and Germ 2009), among others. Our results highlight that, despite of their geographical proximity, ETS activity in sediments of study lakes is controlled by different factors. By using linear regression and multiple regression analysis, OM was identified as the best predictor of ETS in Lake Honda while Chl a was the best predictor in Lake Nueva, being the last one regression weaker than that observed between OM and ETS in Lake Honda.

Table 5 ETS ($\mu l\ O_2\,g^{-1}\,D.W.h^{-1}$) in the upper sediments of different aquatic ecosystems

Lake	ETS	Reference
Honda[a]	17–69	This study
Nueva[a]	21–239	This study
Kriško Sup	165	Muri and Simčič (2004)
Ledvica	75	Muri and Simčič (2004)
Planina	275	Muri and Simčič (2004)
Bled	310	Muri and Simčič (2004)
Strandsjön	311	Broberg (1985)
Ramsjön	319	Broberg (1985)
Erken	211	Broberg (1985)
Orrtjärn	234	Broberg (1985)

[a] Min–Max of ETS in the three sampling stations in the study sites

The strong influence of OM supply on ETS activity in sediment from Lake Honda was the consequence of the labile nature of sedimentary OM. Therefore, and according to Relexans et al. (1992), the higher ETS/OM ratio (a useful indicator of organic carbon quality) in sediments from Lake Honda compared to those from Lake Nueva reflect the more labile nature of the sediment OM. These results are in agreement with those from de Vicente et al. (2003), who found that the ratio between org-P_{acid} (acid soluble organic P) and org-P_{alkali} (alkali soluble organic P), an indicator of the biodegradability of the org-P pool, was lower in Lake Honda (0.31) than in Lake Nueva (0.94), suggesting that the biodegradability of the sediment OM was higher in the former one that showed a lower content of OM in the top sediment. Even more, although the average C/P ratio in settled matter was larger in Lake Honda than in Lake Nueva, the C/P ratio of the top sediment was significantly higher in Lake Nueva (de Vicente et al. 2003); so it is likely that the top sediment of Lake Nueva had a different source of organic matter, and hence C, that increased the C/P ratio. In this sense, the concentration of planktonic Chl a is usually higher in Lake Honda, while the lower turbidity of the water in Lake Nueva favored the growth of submersed macrophytes in the littoral area during spring–summer (Cruz-Pizarro et al. 2002). As it is well known, phytoplankton detritus is easily degradable, while vascular plant remains are structurally complex and their degradation is slower (Kristensen et al. 1995).

By contrast, a large contribution of phytobenthos to ETS has been recognized in sediments from Lake Nueva. The relevance of this community for the whole lake metabolism

was already stated by G.-Tóth (1992), who found that a great proportion of the total primary production in Lake Balaton was comprised by benthic primary production. In Lake Nueva, higher water transparency allows the development of a well-established benthic community which notably contributes to the whole lake metabolism. However, the light availability also favors the presence of vascular plants that grew on the sediment and hence, contribute to the input of refractory OM. In addition, previous studies (Bayo et al. 2003) have revealed that meiofauna in the sediment from Lake Nueva is significantly higher (937± 322 ind m^{-2}) than in Lake Honda (40±14 ind m^{-2}) as a consequence of its lower trophic state. Therefore, it is also likely that apart from phytobenthos, zoobenthos may also contribute to the higher ETS of Lake Nueva compared to Lake Honda.

Finally, and contrary to Relexans (1996b), who found that the ETS provided a good estimate of bacterial activity, our results have shown a weaker relation between aerobic and anaerobic bacteria and ETS in both study sites. One likely explanation may be that, as Trevors (1984) suggested, the number of viable microbial cells in the sediment is much lower than the number of total cells. Moreover, ETS is the result of the activity of both intra and extracellular enzymes, being these ones able to remain active for several days or even weeks (G.-Tóth 1992; G.-Tóth et al. 1994; Trevors 1984).

In summary, the results of this study revealed that the relative importance of planktonic primary producers (phytoplankton), benthic algae and vascular plants in the study sites could explain the differences observed in the intensity of sediment ETS as well as in their drivers.

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