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Is net ecosystem production higher in natural relative to constructed wetlands?

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Abstract

Ecosystem metabolism is an important measure of wetland restoration efficacy, and serves to indicate if the system is capable of processing energetic resources. Despite its value, ecosystem metabolism has rarely been included in monitoring programs. In this study, we aimed to achieve the following objectives: i) compare net ecosystem production (NEP) of constructed vs. natural wetlands; ii) identify the highest NEP rate habitats; and iii) define the main environmental factors regulating NEP in different wetland types. Pelagic and benthic NEP rates and physicochemical features were evaluated in three natural and five constructed wetlands in the Middle Ebro River floodplain (NE Spain). Statistical analyses showed pelagic NEP rates peaked in natural wetlands, which produced up to $187.5 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ compared to lower rates in constructed wetlands (up to $46.2 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$). Pelagic NEP responded positively to temperature, total dissolved solids, and nutrients. Benthic NEP rates were three- to 30-fold greater than pelagic in natural (up to $994.9 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$) and constructed (up to $1551.5 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$) wetlands. Benthic NEP was heavily influenced by habitat type, and NEP peaked in areas dominated by submerged vegetation, and fine organic sediment. Rapid recovery in aquatic communities has been reported, however our study suggests an alteration between biological structure (i.e. biodiversity), and functional processes (i.e. ecosystem metabolism), slowing recovery of degraded floodplain constructed habitats. We therefore strongly advocate the inclusion of ecosystem function in the design and evaluation of restoration projects to optimise long-term wetland ecosystem sustainability.

Keywords: Benthic ecosystem metabolism; dissolved oxygen; ecosystem functions; Generalized Additive Models (GAM); net primary production; pelagic ecosystem metabolism.

Introduction

Wetlands occupy approximately 6% of the world's land surface, and play integral roles in the global carbon cycle by processing carbon, and emitting greenhouse gases (Sahagian and Melack 1998; Ferrati et al. 2005; Erwin 2009). Despite this vital contribution to ecosystem function, wetlands are one of the most threatened and degraded ecosystems in the world (Tockner and Stanford 2002). Since the early 1900's, floodplain wetlands have lost an excess of 50 % of their surface area as a consequence of increasing floodplain occupation, extensive river impoundment, and wetland dredging (Mitsch and Gosselink 2007). Consequently, in recent years wetland restoration and construction has become an important conservation strategy to offset continuous floodplain wetland loss, increase local biodiversity, and treat urban and agricultural waste water (Mitsch and Gosselink 2007; Coveney et al. 2002).

Traditionally, wetland restoration efficiency, and subsequent environmental quality have been analysed by measuring the distribution, abundance, richness, and diversity of plant and animal species (Race 1985; Croonquist and Brooks 1991; Henry and Amoros 1995; Fellows et al. 2006). These ecosystem structure measures are evaluated because they reflect anthropogenic impacts (e.g. dumping, flow control, land use), natural perturbations (e.g. floods, droughts), and fundamental ecological processes (e.g. nutrient cycling, decomposition) on aquatic systems. However, biological structure and ecosystem functioning are not always correlated, and restoration of one element does not necessarily result in recovery of the other (Grayson et al. 1999; Moreno-Mateos et al. 2012). Consequently, ecosystem functioning (e.g. resilience, metabolism, nutrient cycling) indicates which organisms are supported by the system, the interactions among organisms, the physical environment, and the overall services provided by the ecosystem (Mitsch and Gosselink 2007; McKenna 2003; Erwin 2009). Among ecosystem functions, metabolism (i.e. the balance between primary production and community respiration) represents the integrated measure of organic matter production and consumption rates (Odum 1971). Ecosystem metabolism therefore provides a measure of the system's capacity to recycle organic matter and nutrients, process and transfer energy to higher trophic levels, and act as a sink or source for greenhouse gases (Mitsch and Gosselink 2007). Recent studies suggest that to achieve a more comprehensive wetland restoration assessment, in addition to aspects of aquatic community structure, monitoring should evaluate levels of ecosystem function, including ecosystem metabolism (Rapport et al. 1998; Fellows et al. 2006).

Several studies have reported that constructed wetlands support species assemblages similar to natural wetlands (e.g. Mitsch and Gosselink 2007; Gallardo et al. 2008). However, it remains unclear if constructed wetlands operate at metabolic rates similar to natural wetlands. Constructed wetlands exhibit fundamental differences in age, aquatic community development, temperature, light availability, and inorganic and organic nutrient concentrations. Cumulatively, these characteristics have direct and indirect effects on photosynthesis, oxygen solubility, and gas exchange processes, which presumably alter the metabolic rates between natural and constructed wetlands (Petersen et al. 1997; Caraco and Cole 2002; Hanson et al. 2003; Azevedo et al. 2006; Staehr et al. 2010). Reeder (2011) reported that natural wetlands exhibited higher production rates than constructed wetlands. This fact was related to the greater age of natural wetlands, which showed more mature, structured, and diversified aquatic communities, and consequently higher nutrient input from surrounding riparian habitat. However, constructed wetlands can reach high production rates due to low turbidity, which enables light penetration favouring primary production, and low organic matter content that limits ecosystem respiration (Biddanda and Cotner 2002; Azevedo et al. 2006, Lauster et al. 2006). Furthermore, wetlands with increased chlorophyll *a* concentrations might exhibit higher biomass production rates, because this parameter is typically associated with primary producers, mainly phytoplankton and bacterioplankton (Steinman and Lamberti 1996; Hanson et al. 2003). Other habitat related factors, such vegetation presence (e.g. emergent or submerged), and sediment type (e.g. fine or gravel) could lead to differences in ecosystem metabolism between natural and constructed wetlands. Photosynthesis and respiration by aquatic macrophytes, together with decomposition of substantial plant biomass amounts might have a marked influence on ecosystem metabolism (Godshalk and Wetzel 1978; Kaenel et al. 2000). Therefore, it is clear that many varied biological, physical, and chemical factors directly and indirectly exhibit complex interactions, and affect ecosystem metabolism. McKenna (2003) reported that little is known regarding ecosystem production in restored or constructed environments, as most studies have been conducted in natural wetlands. Furthermore, the importance of habitat type in ecosystem metabolism has not been ascertained (McKenna 2003). Due to the potential role of constructed wetlands in the global carbon cycle, the factors affecting the metabolic balance of these constructed ecosystems are critical to the design of multipurpose wetlands. Worldwide, constructed wetlands have become extremely important in various applications ranging from the creation of new or restored wildlife habitat, as a source to treat human waste/sewage water, storm water runoff, land reclamation following mining activities, and mitigation measures to balance natural wetlands lost to development. Therefore, the recreation of fully

functional ecosystems is the goal of constructed wetlands, and various complex interrelated functional ecosystem factors must be elucidated.

In the Ebro River floodplain of NE Spain, a number of wetlands have been constructed in the last two decades for gravel mine habitat restoration and wetland loss mitigation (Gallardo et al. 2012a). Aquatic community changes, sediment, and hydrochemical characteristics were previously analysed in some constructed and natural Ebro floodplain wetlands. Results detected significant differences primarily related to flood frequency, surface and subsurface flows, and habitat successional state (Gallardo et al. 2008, 2012a, 2012b; Cabezas et al. 2008, 2009a, 2009b). Rapid recovery of constructed wetland biodiversity was observed (Gallardo et al. 2008, 2012a); however similar information on recovery of functional aspects is not available. In this study, we aimed to achieve the following objectives: i) compare net ecosystem production (NEP) (pelagic and benthic) of constructed vs. natural wetlands of the Middle Ebro River floodplain; ii) identify the highest NEP rate habitats in each wetland type (constructed vs. natural); and iii) define the main environmental factors regulating NEP in constructed vs. natural wetlands.

Material and methods

Study sites and sampling design

The Ebro is the largest river in Spain, with a length of 910 km and a drainage basin of 85,534 km². Historically, extraordinary flood events in the Ebro River have generated a number of natural wetlands in its floodplain, including oxbow wetlands and temporary pools (Ollero 2007). The Ebro River has been extensively affected by land use changes (agriculture and urban), and construction of structures to control floods, most intensively since the 1960s (Cabezas et al. 2008). These changes have continued to reduce the probability of new natural wetland creation (Gallardo et al. 2012a). A number of artificial wetlands have been constructed in recent years along degraded Ebro floodplain areas to mitigate this habitat loss and increase biodiversity.

For this study, three natural and five constructed wetlands were selected in three different riparian areas of the Middle Ebro River (NE Spain, 41°39'N, 0°52'W; Fig 1, Table 1). Each of the three riparian areas was characterised by the presence of one natural wetland (N1, N2 and N3), and one or two constructed wetlands, no farther apart than 0.8 km: two constructed wetlands (C1a and C1b) in riparian area 1 close to N1; another two (C2a and C2b) located in the vicinity of N2; and only one constructed wetland (C3) near N3 (refer to wetland locations in Fig. 1). Therefore, the study design provided a unique opportunity to investigate constructed wetland

development with close natural wetlands as reference sites, sharing the same local environmental conditions (e.g. light, wind speed, hydrological influences). It should be noted that in this study we use the term ‘reference’ as representative of natural conditions regardless of their environmental quality, as opposed to ‘good reference’ conditions applied in other studies. Cross-comparisons were conducted between each constructed wetland, and its reference natural wetland within each riparian area (e.g. between C1a and N1, or between C2a and N2). The two-three most representative habitats in each wetland were identified, and selected as sampling points to cover the full range of environments available (see sampled habitats in Table 1). Physicochemical characteristics and net ecosystem production (pelagic and benthic) were studied at each sampling point in two seasons: once in winter (December 2010), and once in spring (June 2011). These seasonal measures allowed to incorporate a maximum variation range of environmental conditions throughout the year, with maximum production rates in spring and minimum in winter as reported other studies (Fontaine and Ewel 1981, Tuttle et al. 2008, Sadro et al. 2011). It should be noted that an insufficient water level in C1a prevented physicochemical characteristics, and NEP (pelagic and benthic) measurements for winter sampling. Benthic NEP at C3 was not measured in winter due to excessive depth, however we relied on pelagic NEP records.

Physicochemical characteristics

At each sampling point for both sampling seasons, triplicate water samples were collected directly into 1.5 L acid-washed PVC bottles at a depth of 10 cm, and transported in dark cool-boxes to the laboratory (see total number of water samples in Table 1). Upon arrival, alkalinity of unfiltered water samples was estimated within 4 h of collection by automatic titration with H_2SO_4 0.04 N (APHA 1989). Total suspended solids, total dissolved solids, and organic matter content were determined by the gravimetric method, filtering samples through pre-combusted (450°C, 4 h) Whatman GF/F glass-fibre filters following standard protocols (APHA 1989). Chlorophyll *a* concentration samples were filtered through Whatman GF/F glass-fibre filters, extracted in 96% ethanol for 24 h, and analysed using the spectrophotometric method (Thermo Helios α ; APHA 1989). Filtered water aliquots were stored at -20 °C, and used within one month for remaining analyses. Ion chromatography (Metrohm 861 Advanced Compact IC; APHA 1989) was applied to determine dissolved inorganic nitrogen ($\text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$) and sulfate (SO_4^{2-}). Soluble reactive phosphorous was measured by the ascorbic acid method (APHA 1989). Total dissolved phosphorous was also estimated by the ascorbic acid method, but a previous potassium persulfate digestion was performed (90 min, 115 °C) (APHA 1989). Finally, water

temperature, pH, conductivity, and dissolved oxygen were recorded in situ by portable probes (WTW Multiline P4 and Hach-Lange HQ). Photosynthetic active radiation (PAR) was only measured during the spring season at 5-10 cm below the surface using a sensor (Solar Light Co., PAR detector PMA2132). Due to technical difficulties, data is not available from the winter sampling period; PAR is therefore used only for general comparison between natural and constructed wetlands, and not directly included in calculations.

Pelagic and benthic net ecosystem production (NEP)

Pelagic and benthic NEP rates were measured within the aquatic environment as representatives of different ecosystem metabolism components. Net ecosystem production (NEP) corresponds to the balance between gross inorganic carbon fixation by photosynthesis (Gross Primary Production, GPP), and organic carbon remineralised to CO₂ by all organisms of the ecosystem (Community Respiration, CR) (Laas et al. 2012; Staehr et al. 2012a).

Pelagic NEP represents the productivity of the water column, and is supported by phytoplankton, zooplankton, and bacterial communities. In contrast, benthic NEP is an integrated contribution of the water column and sediment, including metabolic activity of macrophytes, macro- and microalgae, invertebrates, and microbial communities (Gazeau et al. 2005; Staehr et al. 2012a, 2012b).

Pelagic NEP was estimated using an incubation bottles method (Wetzel and Likens 1991; Reeder and Binion 2001; Lauster et al. 2006). For both sampling seasons, triplicate light bottles (0.25L Winkler bottles) were located in situ, floating at 5-10 cm below the upper water layer of each sampling point (6-9 bottles per wetland and season, see Table 1) for a 5-6 h period. We assumed pelagic production and respiration occurs at this depth in the un-stratified shallow wetlands. Initial (t = 0 h) and final (t = 6 h) dissolved oxygen concentration was measured in each bottle using a portable sensor (Hach-Lange HQ, range: 0.1 to 20.0 mg/L).

Likewise, benthic NEP was determined by measuring dissolved oxygen concentration change within light chambers over a 5 - 6 h period (Carignan 1998; Fellows et al. 2006; Sadro et al. 2011). Chambers were made of colourless methacrylate with the following dimensions: 35 x 30 x 30 cm, volume = 36 L, wall thickness = 5 mm. The chamber sidewall had an upward facing hole with a stopper, where a temperature and dissolved oxygen sensor was placed. The chambers did not measure water circulation as is typical in other studies, because water flow at the study sites was imperceptible, and some habitats supported large vegetation masses (Velasco et al. 2003). For both sampling seasons, three light chambers were placed on the substrata at similar depths (50 – 70 cm) at each sampling point (6-9 chambers per wetland and season, see Table 1), so calculations would not be

biased by depth. Light penetrated the bottom sediment at all sampling points, which was verified with a Secchi disk.

Changes in dissolved oxygen concentration were measured to obtain pelagic and benthic NEP rates. Light bottles and chambers were respectively used for pelagic and benthic zones, and corrected for water volume incubation and time (units were $\text{mg O}_2 \cdot \text{m}^{-3} \cdot \text{h}^{-1}$). Benthic NEP rates were also corrected for enclosed surface and incubation time (units were $\text{mg O}_2 \cdot \text{m}^{-2} \cdot \text{h}^{-1}$). The NEP rates were converted to carbon units assuming that one mole of C is equivalent to one mole of O_2 for photosynthesis (e.g. $1 \text{ mg O}_2 = 0.375 \text{ mg C}$, Fellows et al. 2006). Reported NEP rates corresponded to standard measures per cubic meter (m^3), so metabolic rates could be compared under similar conditions. However, natural wetlands were notably larger in size than constructed wetlands (Table 1). We had to consider that when values were scaled up to the entire wetland area occupied by each habitat, NEP rates had the potential to increase. Therefore, NEP rates were interpolated over the entire wetland using percentage habitat coverage values calculated from digitised aerial photographs in ArcView 9.3 ([©]Esri, Redlands, CA). These calculated whole ecosystem measures illustrated the potential role of natural and constructed wetlands as carbon sinks or sources, and allowed comparisons with published data, which was typically in this form.

Statistical analyses

All variables except pH were $\log(x + 1)$ transformed to normalise distributions, and linearise relationships. Because data continued to show a non-normal distribution (Kolmogorov-Smirnoff test, $p < 0.05$), non-parametric Mann-Whitney U-tests (for two samples), and Kruskal-Wallis tests (for k samples) were applied to identify significant differences in physicochemical features and metabolic rates as follows: i) between natural and constructed wetlands within each riparian area (e.g. C1a vs. N1, and C1b vs. N1 in riparian area 1; C2a vs. N2 in riparian area 2, and so on); and ii) among habitats (substrata with emergent vegetation: *Typha* sp. and *Phragmites* sp; submerged vegetation: *Chara* sp.; in the absence of vegetation: fine and gravel sediments). Non-parametric analysis of variance (ANOVA) and correlation analysis were performed using SPSS version 18.0 ([©]SPSS, Inc., Chicago). Subsequently, Generalized Additive Models (GAMs; Hastie and Tibshirani 1990) were used to model the pelagic and benthic NEP rate response to physicochemical features. Only non-correlated (Spearman rank $r < 0.8$) parameters were evaluated in the models, which were run separately for natural and constructed wetlands. In this way, differences in the main drivers controlling pelagic and benthic NEP could

potentially be identified. A stepwise procedure was applied to select significant predictor variables (a priori P-value < 0.05) for each model, until all predictors were significant, i.e. “optimized model”. The explained deviance (D^2) from each final model was compared to assess model goodness-of-fit. The importance of each variable in the optimised model was evaluated using a jackknife approach, where models were sequentially run removing one variable at a time (“model following variable removal”). In addition, models were also run including only one variable at a time to analyse its single potential contribution (“model including independent variable”). These two complementary procedures allowed identifying variables contributing most to the model individually, and in combination with the remainder of the predictors. GAMs were performed using the “mgcv” package (Wood 2011) in R version 2.12.2 (R Development Core Team, 2007).

Results

General physicochemical characteristics of natural and constructed wetlands

The chlorophyll *a* concentration (Mann-Whitney U-test, $Z = -6.87$, $P < 0.01$), organic matter ($Z = -4.92$, $P < 0.01$), soluble reactive phosphorous ($Z = -5.41$, $P < 0.01$), and total dissolved phosphorous ($Z = -5.67$, $P < 0.01$) were more prominent in natural wetlands (Table 2). Among habitats, total dissolved solids (Kruskal-Wallis test, $X^2 = 22.8$, $P < 0.01$) and sulfate ($X^2 = 16.96$, $P < 0.01$) concentration were highest in habitats dominated by submerged vegetation (*Chara* sp.), and lowest in those with emergent vegetation (*Phragmites* sp.). Soluble reactive phosphorous concentration ($X^2 = 18.19$, $P < 0.01$) peaked in habitats with fine organic sediment that lacked vegetation. In addition, total suspended solids ($X^2 = 14.67$, $P < 0.01$), organic matter ($X^2 = 11.34$, $P < 0.05$), and chlorophyll *a* ($X^2 = 15.19$, $P < 0.01$) were habitats exhibiting gravel sediments and submerged vegetation (*Chara* sp.).

Net ecosystem production in natural and constructed wetlands

Analysis by riparian area showed pelagic NEP rates were higher in natural than constructed wetlands ($N1 > C1a$, $Z = -2.73$, $P < 0.01$; $N1 > C1b$, $Z = -2.53$, $P = 0.01$; $N2 > C2a$, $Z = -3.64$, $P < 0.01$; $N2 > C2b$, $Z = -3.15$, $P < 0.01$), with the exception of riparian area 3, where $N3$ and $C3$ exhibited similar NEP rates. Benthic NEP rates were only significantly higher in $N2$ than $C2b$ ($Z = -2.61$, $P < 0.01$), while no significant differences were observed in other riparian wetland areas. Benthic NEP rates were three- to 30-fold higher than pelagic in both wetland types (Table 2). Results indicated $N2$ had the highest productivity for pelagic ($187.5 \text{ mgC} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$) and

benthic ($994.9 \text{ mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$) zones (Table 2), however the lowest benthic NEP rates ($-91.4 \text{ mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$) were recorded for N2, and the lowest pelagic NEP values were observed in C3 ($-27.7 \text{ mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$). Following interpolation of values to the entire ecosystem using habitat coverage, total pelagic NEP rates for natural wetlands ranged from 10.2 to $50.9 \text{ kg C}\cdot\text{h}^{-1}$, and total benthic NEP rates ranged from 13.0 to $250.0 \text{ kg C}\cdot\text{h}^{-1}$. In contrast, constructed wetland production was markedly lower due to the smaller sized constructed wetland area; total pelagic NEP rates ranged from 0.03 to $0.38 \text{ kg C}\cdot\text{h}^{-1}$, and total benthic NEP rates ranged from 0.12 to $2.61 \text{ kg C}\cdot\text{h}^{-1}$.

Net ecosystem production in different wetland habitats

The highest average pelagic and benthic NEP rates in natural wetlands were found in habitats dominated by fine organic sediments that lacked vegetation (average $71.8 \pm 67.1 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ for pelagic NEP; and $299.9 \pm 318.1 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ for benthic NEP). The highest NEP rates in constructed wetlands were observed in habitats with submerged vegetation, particularly dominated by *Chara* sp. (pelagic NEP = $14.9 \pm 20.8 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$; and benthic NEP = $618.7 \pm 547.9 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$), and habitats dominated by fine organic sediments (pelagic NEP = $18.7 \pm 10.6 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$; and benthic NEP = $115.9 \pm 112.8 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$). In areas dominated by emergent vegetation, specifically *Typha* sp., results revealed the lowest NEP rates in natural (pelagic NEP = $18.4 \pm 6.7 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$; and benthic NEP = $-79.9 \pm 11.5 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$), and constructed (pelagic NEP = $3.1 \pm 15.8 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$; and benthic NEP = $138.9 \pm 150.3 \text{ mg C}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$) wetlands.

Fine organic sediments alone from the cumulative area occupied by each habitat contributed to approximately 55-75 % of the carbon production in natural wetlands in the pelagic and benthic zones (pelagic NEP = 1.5 to $40.0 \text{ kg C}\cdot\text{h}^{-1}$; and benthic NEP = 5.3 to $200.0 \text{ kg C}\cdot\text{h}^{-1}$). The contribution of submerged vegetation in constructed wetlands reached 50 % of the total carbon production (pelagic NEP = 0.01 to $0.06 \text{ kg C}\cdot\text{h}^{-1}$; and benthic NEP = 0.20 to $1.78 \text{ kg C}\cdot\text{h}^{-1}$), despite the overall low area coverage of this habitat (12 %).

Environmental factors controlling net ecosystem production

Generalized Additive Models (GAM) showed a significant relationship between NEP and various physicochemical parameters; and a distinct response was detected between natural vs. constructed wetlands, and pelagic vs. benthic zones (Table 3, Fig. 4). In constructed wetlands, pelagic NEP was positively associated with water temperature (Temp), pH, organic matter (OM), and dissolved inorganic nitrogen concentration (DIN).

GAM results indicated temperature (Temp) was the most important variable, with a 12.7 % drop in deviance when removed from the model; in addition, temperature explained as much as 47.7 % of the deviance when used independently (Fig. 4). Interestingly, organic matter (OM) and chlorophyll *a* (Chla) exhibited a minor decrease in deviance when removed from the pelagic model (3.6 % and 4.3 %, respectively, Fig. 4). In comparison, N3 chlorophyll *a* (Chla) concentration was four-fold higher than C3, even though their NEP rates were similar. Benthic NEP was positively related to water temperature (Temp), pH, total suspended solids (TSS), total dissolved solids (TDS), and habitat type (Habitat). The latter explained up to 44.6 % when used independently, and caused a 29.0 % deviation decrease when excluded from the model (Fig. 4). In natural wetlands, pelagic NEP was positively associated with total dissolved solids (TDS) concentration, which resulted in a 28.3 % deviance decrease when removed from the model, and explained 47.7 % of pelagic NEP variability when used independently (Fig. 4). NEP was positively related to water temperature (Temp), total dissolved solids (TDS) concentration, and habitat type (Habitat) in the benthic zone, which indicated the highest production rates from wetland sites of fine organic sediment, and lowest rates from *Typha* sp. dominated habitats. Dissolved inorganic nitrogen (DIN), and total dissolved solids (TDS) served to explain more deviance when modelled independently (46.4 % and 46.3 %, respectively), while total dissolved solids (TDS) also resulted in a notable drop in deviance when removed from the analysis (23.1 %, Fig. 4).

Discussion

Is net ecosystem production higher in natural relative to constructed wetlands?

The results of our study suggest that natural wetlands tend to register much higher NEP rates than constructed wetlands. Pelagic NEP rates in natural wetlands peaked between 10 to 50 kg C h⁻¹, relative to rates lower than 1 kg C h⁻¹ in constructed wetlands. Benthic NEP rates were five- to 2000-fold greater than pelagic NEP rates in natural (13-250 kg C h⁻¹) and constructed (0.1-3 kg C h⁻¹) wetlands. These values refer to the entire wetland area, and standard NEP rates per m³ were not statistically different between natural and constructed wetlands, suggesting benthic ecosystem production recovers more rapidly from restoration. Low NEP rates observed in constructed ecosystems were inconsistent with a rapid recovery in the system's biological structure (Gallardo et al. 2012a). Gallardo et al. (2012a) reported that macroinvertebrate abundance and richness in a constructed wetland reached and exceeded values relative to a natural wetland within eight months following construction, which was related to the provision of new habitats (e.g. submerged vegetation, gravel sediment) for novel

species that rapidly dispersed to other local wetlands, thereby increasing local biodiversity. Therefore, a disparity exists between the recovery of biodiversity, which can be rapid, and other ecosystem functions, such as ecosystem metabolism, which can be slow. A global meta-analysis performed by Moreno-Mateos et al. (2012) demonstrated that structural aspects (e.g. faunal assemblages and physicochemical characteristics) recover more efficiently in restored habitats than functional aspects (e.g. ecosystem metabolism, carbon and nitrogen storage and cycling), which often do not reach reference conditions, congruent with the above observations. This decoupling was attributed to two non-exclusive hypotheses: (i) restored ecosystems require more time (> 30 years) to reach similar functional characteristics as reference ecosystems that are generally more mature and stable; and (ii) restored wetlands are dynamic ecosystems shifting to alternative states not necessarily similar from the reference ecosystem, which would confer the restored sites certain flexibility or resilience to perturbations. Constructed wetlands investigated in this study are relatively young (5 to 25 years), and therefore have had less time to accumulate and produce organic matter and nutrients than more mature natural wetlands (50 to 65 years). To illustrate this, the oldest studied constructed wetland (C2a, 25 years) showed greater organic matter, nutrient concentration and benthic NEP rates than a younger constructed wetland (C2b, six years) located in the same riparian area (Table 2, Fig. 2). Thus, NEP rates in constructed wetlands (e.g. benthic NEP = 159 ± 56 $\text{mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ in C2a) may increase over time, and eventually equal rates of nearby natural wetlands (e.g. benthic NEP = 317 ± 400 $\text{mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ in N2). Ecosystem age was also emphasised by Reeder (2011), where despite recording similar high NEP rates ($86\text{-}109$ $\text{mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$) to our study in constructed wetlands, values were notably lower than obtained in reference natural wetlands. However, age is not the only factor dictating differences in the ecosystem production, as shown in lower pelagic NEP rates in C2a (25 years) compared to N2 (50 years). Differences in riparian vegetation adjacent to wetlands may provide a plausible explanation for differences in ecosystem production. González et al. (2010) reported herbaceous vegetation detritus in constructed wetlands generally provided decreased organic matter and nutrients relative to the leaf litter and wooden detritus provided by natural wetlands. Four years following wetland restoration, McKenna (2003) recorded similar metabolic rates in constructed and natural wetlands, which was linked to riparian habitat development that provided similar organic matter and nutrients in both wetland types. Morphometric differences (e.g. surface area and depth) may also explain a lack in functional aspect recovery reported in constructed wetlands, which have been emphasised as integral in metabolic balance regulation (Mitsch and Gosselink 2007; Hanson et al. 2007; Staehr et al. 2012b). Finally, hydrology can be a substantial influence of wetland communities. Floods introduce large amounts of

nutrients and sediments, and reset floodplain wetland aquatic communities (Cronk and Mitsch 1994; Gallardo et al. 2012b). According to a recent study, Gallardo et al. (2012b) found flood pulses in two natural wetlands boosted productivity rates in response to a change in water physicochemical conditions, and replacement of autotrophic phytoplankton by other autotrophic organisms.

Environmental factors controlling metabolic balance

Statistical models revealed that metabolic rates were mainly controlled by habitat type, and physicochemical factors such as temperature and nutrient concentration, were directly and indirectly linked to photosynthesis, respiration, and gas diffusion processes. A major predictor variable of wetland metabolic response was habitat type, overall in the benthic zone. Among habitats, our results suggested submerged vegetation is a hot-spot of carbon production ($618.7 \pm 547.9 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$), indicating large portions of the carbon in constructed wetlands is produced by benthic submerged macrophytes, rather than suspended phytoplankton, as suggested elsewhere for aquatic ecosystems with low turbidity (Kaenel et al. 2000; Lauster et al. 2006). In natural wetlands, light penetration in unvegetated habitats, and nutrient accumulation in the upper sediment favoured phytoplankton productivity (Watt and Golladay 1999; McKenna 2003; Reeder 2011), resulting in some of the highest NEP rates. In contrast, habitats dominated by emergent vegetation (*Typha* sp.) exhibited six-fold lower values. The photosynthetic products of emergent vegetation are released outside water, therefore the main interchange of oxygen and carbon fixation was not reflected in the pelagic and benthic measures obtained in this study (Ibañez et al. 1999; Scarton et al. 2002; Vis et al. 2007). In addition, organic carbon accumulated around emergent macrophytes may further explain the oxygen loss reported in this study ($-79.9 \pm 11.5 \text{ mg C} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ pelagic NEP in *Typha* sp. dominated habitats), via heterotrophic respiration, as noted by Lauster et al. (2006). Nutrients (nitrogen and phosphorous compounds), and organic matter were also important drivers of metabolic balance. The observed negative relationship between metabolic rates and dissolved inorganic nitrogen indicated this fundamental element may have been in excess; consequently results suggested phosphorous was a limiting ecosystem production factor. The low deviance explained by both nitrogen and phosphorus suggested the nutrient importance in metabolic balance was lower than demonstrated in other studies (e.g. Cole et al. 2000; López-Archilla et al. 2004), although congruent with Gallardo et al. (2012b) where nutrients and organic matter concentration exhibited little effect on production rates.

Abiotic factors, including temperature, pH, and total dissolved solids increased NEP rates in natural and constructed wetlands, consistent with results from previous studies (Demars et al. 2011; Gallardo et al. 2012b). Temperature and high pH favoured CO₂ diffusion, and metabolic activity activation (Lopez-Urrutia et al. 2006; Yvon-Durocher et al. 2010). Furthermore, high system pH may also result from increased algae and macrophyte primary production that decreased CO₂ from the system (Racchetti et al. 2010). Total dissolved solids provided essential nutrients for primary producers (Cole et al. 2000; McKenna 2003; Fellow et al. 2006), which acted as a limiting factor in the metabolic process, especially in wetlands with low solid concentrations (e.g. C2b, C3 and N3). An additional factor found to control metabolic activity was turbidity (total suspended solids) caused by bottom sediment re-suspension, which has been reported to hinder light penetration aquatic environments (Sand-Jensen 1983; Kaenel et al. 2000).

Apart from factors explored in this study, other researchers have noted dissolved organic carbon as an important factor inhibiting ecosystem metabolism, which typically reduces light penetration and planktonic production, while increasing respiration by plankton and organic matter mineralization (del Giorgio and Peters 1994; Hanson et al. 2003; Staehr et al. 2010). In addition, sediment characteristics including organic matter content, redox capacity, and light availability could have been integral factors of benthic ecosystem metabolic regulation (Pinardi et al. 2011; Staehr et al. 2010, 2012a).

NEP rates recorded in this study may have been underestimated due to limitations associated with the gas flux effects between the water-atmosphere interface, and horizontal-vertical gas dispersion in water (Chen et al. 2000; Lauster et al. 2006; Van de Bogert et al. 2007). Pelagic NEP was only measured in the wetland upper mixed layer, and extrapolated to the entire water column, which assumed the absence of a vertical distribution of chlorophyll *a*, and that most production occurs in this upper layer. In terms of benthic measures, due to dimension limitations, our incubation chambers could not be used in the deepest regions of our studied wetlands. Moreover, habitats dominated by submerged vegetation were only found in constructed wetlands, limiting comparisons of metabolic rates with natural wetlands.

Applications for wetland restoration

Positive NEP rates recorded in Ebro floodplain natural and constructed wetlands suggest that both systems are short-term carbon sinks fed by autochthonous organic matter and allochthonous terrestrial inputs (McKenna 2003; Demars et al. 2011). Furthermore, Cabezas et al. (2009b) demonstrated higher carbon and nitrogen sediment

accumulation rates in mature natural wetlands of the Ebro floodplain than in other aquatic systems, including peatlands, mangroves, and marshes (Cabezas et al. 2009b). The floodplain wetland capacity as a carbon sink should be regarded as an additional positive outcome of restoration projects, and worthy of encouragement. At least four recommendations can be made to introduce functional aspects during restored/constructed wetland project design and evaluation to optimise efficient recovery of important ecosystem functions, such as ecosystem metabolism and carbon storage. First, our results suggest that wetlands with increased area and depth have higher net ecosystem production. Therefore, restoration objectives should have preference for larger wetland areas, although it remains unclear if one large constructed wetland, or several smaller wetland areas are more efficient to store carbon (Staehr et al. 2012b). Consequently, morphometric aspects including size, shape, and depth should be further explored to optimise new constructed wetland design. Second, ecosystem production measures such as those offered in the present study, can be useful to identify habitats acting as hotspots of productivity; here submerged vegetation and fine organic sediments were identified, which can be promoted in restoration projects (McClain et al. 2003). Third, it is critical to consider communities interacting with the wetland, most notably the adjacent riparian community development, which can be key to provide the necessary organic matter and nutrients to encourage metabolic activity (McKenna 2003; González et al. 2010). Fourth, increasing the hydrological connectivity of restored wetlands with the main river channel will serve to promote metabolic carbon storage (Cronk and Mitsch 1994; Gallardo et al. 2012b), and equally important, enhance aquatic biodiversity (Gallardo et al. 2008), riparian vegetation development (Cabezas et al. 2008; González et al. 2010), and biogeochemical cycling (Cabezas et al. 2009a, 2009b). Finally, it is vital to perform periodic assessments to record possible changes in productivity trends, as metabolic rates experience wide temporal fluctuations related to seasonality and disturbance events (e.g. wind storms, floods) that can strongly influence annual restored habitat balance (Gallardo et al. 2012b). This study and others have clearly demonstrated the indispensable role of floodplain wetlands in the carbon cycle. Therefore, policy-makers and stakeholders should promote the incorporation of functional aspects into wetland management strategies, and advance the recreation of efficient and effective multipurpose wetlands, which in the long term will compensate for the unfortunate loss of ecosystem services observed in the last several decades.

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Table 1. Natural and constructed wetland characteristics. (N = total number of water samples and ecosystem metabolism measures taken in each wetland during the study period – winter 2010 and spring 2011).

Riparian area	Wetland	Type	Age ¹ (years)	Area ² (Ha)	Depth ³ (m)	Substrate	Habitat sampled	N
1	C1a	Const.	7	0.58	0.5	Silt and gravel	Fine sediment, Gravel sediment, <i>Typha</i> sp.	9 ^(a)
	C1b	Const.	5	0.5	1	Silt	Fine sediment, <i>Typha</i> sp., <i>Chara</i> sp.	18
	N1	Natural	65	10.33	2.5	Silt	Fine sediment, <i>Phragmites</i> sp.	12
2	C2a	Const.	25	0.38	1.5	Gravel	Gravel sediment, <i>Chara</i> sp.	12
	C2b	Const.	6	0.84	2	Silt	Fine sediment, <i>Phragmites</i> sp.	12
	N2	Natural	50	70.3	0.8	Silt	Fine sediment, <i>Phragmites</i> sp., <i>Typha</i> sp.	18
3	C3	Const.	15	0.94	1.7	Silt	Fine sediment, <i>Phragmites</i> sp., <i>Typha</i> sp.	9-18 ^(b)
	N3	Natural	65	35.45	2	Silt	Fine sediment, <i>Phragmites</i> sp.	12

¹Age since first observance according to Cabezas et al. (2008)

²Area according to digitalised aerial photographs

³Depth measured along a transect from shore to centre (average value)

^(a) C1a was only sampled in spring 2011.

^(b) C3 was sampled in winter 2010 and in spring 2011 (N = 18 samples), with the exception of benthic NEP rates that were only measured in winter (N = 9 samples).

Table 2. Physicochemical features and net ecosystem production rates (mean $\pm$ SD) in three natural and five constructed wetlands. Parameters in bold show significant differences between constructed and natural wetlands ($P < 0.05$, Mann-Whitney U-test). N = total number of water samples taken in each wetland during the study period.

	Abbr.	Units	Area 1			Area 2			Area 3	
			C1a (N=9)	C1b (N=18)	N1 (N=12)	C2a (N=12)	C2b (N=12)	N2 (N=18)	C3 ^(a) (N=9-18)	N3 (N=12)
Temperature	Temp	(°C)	18.20 ± 0.31	17.07 ± 7.20	16.03 ± 10.44	19.83 ± 8.36	17.08 ± 11.11	16.61 ± 9.62	12.56 ± 5.64	14.07 ± 5.28
pH	pH		7.23 ± 0.10	8.04 ± 0.28	8.07 ± 0.09	7.72 ± 0.14	8.00 ± 0.11	8.02 ± 0.22	8.10 ± 0.13	7.72 ± 0.24
Alkalinity	Alk	(mg/L)	292.22 ± 9.81	152.67 ± 62.98	167.91 ± 31.88	253.13 ± 19.81	163.43 ± 5.65	134.31 ± 26.56	156.39 ± 3.60	250.54 ± 13.27
Total suspended solids	TSS	(mg/L)	42.42 ± 26.55	20.35 ± 10.38	38.89 ± 6.55	8.70 ± 4.22	24.03 ± 31.05	104.43 ± 87.02	18.46 ± 11.01	10.26 ± 3.64
Total dissolved solids	TDS	(mg/L)	4245.8 ± 39.3	6645.1 ± 566.7	2428.9 ± 737.2	2117.0 ± 128.1	775.2 ± 120.9	2619.6 ± 1590.6	873.7 ± 193.4	1296.0 ± 96.9
Chlorophyll a	Chla	(µg/L)	5.29 ± 2.40	3.29 ± 2.91	29.34 ± 3.83	3.10 ± 1.08	4.65 ± 0.92	45.48 ± 53.37	2.91 ± 1.25	13.30 ± 21.59
Organic matter	OM	(mg/L)	8.40 ± 3.29	6.81 ± 4.19	27.17 ± 2.55	2.75 ± 1.19	5.00 ± 3.83	14.34 ± 7.30	3.11 ± 1.63	3.02 ± 1.06
Dissolved inorganic nitrogen	DIN	(mgN/L)	4.90 ± 1.12	0.59 ± 0.43	1.30 ± 1.75	0.16 ± 0.21	0.31 ± 0.37	0.20 ± 0.21	1.10 ± 0.64	4.61 ± 0.59
Sulfate	SO4	(mg/L)	1834.8 ± 49.8	1187.4 ± 416.7	951.8 ± 579.7	669.9 ± 27.8	158.9 ± 43.1	720.8 ± 467.4	208.7 ± 12.3	299.1 ± 24.0
Soluble reactive phosphorous	SRP	(µgP/L)	1.68 ± 0.26	1.33 ± 1.43	2.03 ± 0.82	0.75 ± 0.35	1.41 ± 0.80	2.08 ± 0.62	0.80 ± 0.46	2.34 ± 1.33
Total dissolved phosphorous	TDP	(µgP/L)	5.51 ± 0.66	7.98 ± 4.76	58.36 ± 31.73	5.60 ± 1.19	4.29 ± 0.71	10.03 ± 1.24	5.10 ± 1.47	5.71 ± 2.71
PAR	PAR	(µE/m ²)	308.1 ± 161.1	1001.3 ± 272.5	396.7 ± 465.1	996.6 ± 166.2	206.8 ± 119.0	361.1 ± 96.8	230.6 ± 211.1	141.1 ± 54.6
Pelagic net ecosystem production	Pelagic NEP (NEPw)	(mgC/m ³ ·h)	15.1 ± 5.0	11.4 ± 17.5	52.7 ± 49.2	10.6 ± 5.6	18.0 ± 13.0	76.2 ± 68.2	7.0 ± 15.0	7.8 ± 7.7
Benthic net ecosystem production	Benthic NEP (NEPc)	(mgC/m ³ ·h)	144.4 ± 46.0	410.0 ± 453.1	142.3 ± 51.6	159.0 ± 56.0	16.9 ± 14.5	317.1 ± 400.1	94.7 ± 82.3	108.3 ± 50.4
		(mgC/m ² ·h)	57.8 ± 14.4	164.0 ± 181.2	56.9 ± 20.6	63.6 ± 22.4	6.8 ± 5.8	126.8 ± 160.1	37.9 ± 32.9	43.3 ± 20.2

^(b) In C3, N = 18 samples to physicochemical characteristics and pelagic NEP measurements, and N = 9 samples to benthic NEP measurements.

Table 3. Response of pelagic and benthic net ecosystem production (NEPw and NEPc, respectively) to habitat and physicochemical parameters according to Generalized Additive Models (GAM). P = variable P - value: *** < 0.001; ** < 0.01; * < 0.05. AIC = Akaike Information Criterion. D2 = total deviance explained by the model. Habitat: Ty- *Typha* sp.; Phrg – *Phragmites* sp.; Ch – *Chara* sp.; FS – Fine Sediment; GS – Gravel Sediment. N = total number of water samples obtained in each wetland type during the study period. See Table 2 for variable abbreviations.

	Natural Wetlands				Constructed Wetlands			
	NEPw (N=42)		NEPc (N=42)		NEPw (N=69)		NEPc (N=60)	
	F	P	F	P	F	P	F	P
Intercept	23.6	***	-11.0	***	15.2	***	13.2	***
Temp			26.8	***	8.6	***	22.3	***
pH			65.7	***	10.4	***	36.7	***
TDS	73.4	***	285.3	***			6.0	**
TSS					7.5	***	9.0	**
OM					3.5	*		
Chla					4.5	**	4.7	*
TDP	8.1	***						
DIN			151.6	***	4.2	*		
SO4	41.6	***			8.8	***	8.2	***
Habitat-Ty			-2.9	***			-6.7	***
Habitat-Phrg			18.9	***			-5.5	***
Habitat-Ch							16.7	***
Habitat-FS			22.0	***			8.4	***
Habitat-GS							12.9	***
Model	Estimate = 0.107 AIC = -203.7 D ² = 93.3%		Estimate = -0.376 AIC = -142.63 D ² = 99.5%		Estimate = 0.031 AIC = -362.16 D ² = 82%		Estimate = 0.212 AIC = -169.16 D ² = 98.2%	

Figure 1. Study site location. Riparian areas: Area 1, Area 2, and Area 3. Natural wetlands: N1, N2, and N3. Constructed wetlands: C1a, C1b, C2a, C2b, and C3. Black dots correspond to wetland sampling sites.

Figure 2. Pelagic and benthic net ecosystem production (NEP_w and NEP_c, respectively) in three natural and five constructed wetlands. Results from non-parametric ANOVA (Kruskal-Wallis test) between natural and constructed wetlands are shown in the upper right corner of each graphic. *** = Significant differences between pairs of wetlands ($P < 0.001$, Mann-Whitney U-test). Grey circles show outliers.

Figure 3. Pelagic and benthic net ecosystem production (NEP_w and NEP_c, respectively) in different habitats of three natural and five constructed wetlands. A: Pelagic zone; B: Benthic zone. Differences in NEP rates between habitat types were non-significant in the pelagic zone. In the benthic zone, NEP rates were only significantly different in submerged vegetation ($P < 0.05$; Mann-Whitney U-test). Grey circles show outliers.

Figure 4. Results of Generalized Additive Models (GAM) used to evaluate the response of pelagic and benthic net ecosystem production (NEP_w and NEP_c, respectively) to physicochemical parameters. A-B: pelagic NEP model in constructed and natural wetlands. C-D: benthic NEP model in constructed and natural wetlands. Model: % total deviance explained by the optimised model. Variable in: deviance explained by the model including the variable independently. Variable out: deviance explained by the model following variable removal. See Table 2 for variable abbreviations.