

Microhabitat patterns of soft-bodied benthic algae in a lowland river largely fed by groundwater

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Abstract: In lowland rivers largely fed by groundwater, the aquifer resurgence plays a major role in supporting local plant diversity. However, large knowledge gaps exist with regard the role of groundwater on algal diversity and spatial arrangement. In this context, our main aim was to evaluate the distributive gradients of soft-bodied benthic algae across a mid-size lowland river with a bottom dominated by hard substrate (Oglio River, north Italy). Along the investigated river sector, a clear segregation both for taxa and for environmental data was observed, especially in terms of pH, hydrological variables and nutrient availability. Overall, we recorded an important contribution of groundwater supply in shaping the structure and complexity of algal communities at the microhabitat level (in the meter range) during the river base flow. All these considerations shed new light on the response of algal taxa to a relevant groundwater ingression in a lowland river.

Key words: algae, cyanobacteria, groundwater dependent ecosystems, spatial distribution, species diversity, hydrology, ecological determinants, nutrients

INTRODUCTION

The correct measurement and evaluation of distributive patterns of species represent a focal point in order to move from correlative to mechanistic relationships linking species diversity or abundance and environmental drivers (GELFAND et al. 2006). This is especially true for aquatic habitats, where the collection of data is very expensive and time-consuming (PASSY & BLANCHET 2007), and it is even more for systems completely or partially fed by groundwater that exhibited typical isolated and point distributions and peculiar physico-chemical mosaic conditions (LAINI et al. 2011).

All these systems are regarded as groundwater dependent ecosystems (GDEs) and include the aquatic water bodies whose extent and dynamics are dependent on groundwater supply, for example springs or wetlands originated by the emergence of groundwater aquifers (KLØVE et al. 2011). GDEs play central roles in supporting both terrestrial and aquatic flora and fauna, especially during prolonged dry periods (KLØVE et al. 2014; KUGLEROVÁ et al. 2014). This fundamental function (the provision of temporary refugees, often for rare or endemic taxa) is expected to be exacerbated and, at the same time, threatened by climate change (CANTONATI et al. 2012; KLØVE et al. 2014; JYVÄSJÄRVI et al. 2015). In this context, acquire information on the

potential role of groundwater supply in supporting the algal diversity and spatial rearrangement could be of fundamental importance to outline plans for the preservation and enhancement of water use under different climatic scenarios.

Furthermore, despite the pivotal role played by algal communities in driving the river primary production, fundamental aspects such as species-area relationships, beta-diversity, and their interaction with habitat characteristics are still unexplored (PASSY & BLANCHET 2007). This is especially true for the soft-bodied benthic algae of rivers, for which the relative importance of environmental factors in microhabitat structuring of their communities is yet largely missing (SCHNEIDER et al. 2013; ROTT & SCHNEIDER 2014). In this regard, a robust scientific literature is available only for diatoms (PASSY 2007; KOLLÁR et al. 2015). Besides, given the central role assigned to the primary producer communities in the ecological assessment of aquatic ecosystems, these knowledge gaps can affect very negatively the quality evaluation of water bodies that is frequently done with limited sampling effort (LAINI et al. 2014).

Focusing on a mid-size river largely fed by groundwater, the resurgence of the aquifer could result in a clear physico-chemical zonation along the lateral gradient, with possible cascading effects on algal alpha-diversity and dominant taxa. We hypothesize that

the transition from the riparian sectors (directly influenced by the groundwater resurgence) to the central river bed area (mainly affected by the river outflow) will deeply shape the composition and structure of algal communities. To test this prediction, an evaluation of the distribution and abundance gradients of soft-bodied benthic algae across a mid-size lowland river with a bottom dominated by hard substrate (Oglio River, north Italy) was performed. Specifically, we assessed the spatial arrangement of soft-bodied benthic algae across the entire riverbed in order to highlight the existence of floristic patterns and major physico-chemical gradients.

MATERIAL AND METHODS

Study site. We collected data from the Oglio River (Lombardia, northern Italy), a mid-size man-regulated river with a total length of 154 km and a historical average discharge of $\sim 80 \text{ m}^3 \cdot \text{s}^{-1}$. It originates from the subalpine Lake Iseo (185 m a.s.l.) and flows into the Po River (16 m a.s.l.) crossing a heavily impacted catchment (of 3,840 km²), mainly devoted to agricultural activities (67%) and animal farming (about 600,000 cows and over 2,100,000 pigs) (BOLPAGNI & PIOTTI 2015, 2016). Consequently, both surface and groundwaters were affected by appreciable nitrogen concentrations (LAINI et al. 2011; SOANA et al. 2011). We focused our attention to the central rhithral sector of this river (of about 20 km) that is characterized by relevant groundwater resurgence (Fig. 1). During summer time, in concomitance with an intense water abstraction for irrigation purposes, along this river sector the resurgence of groundwater represents about the 50% of the water discharge: a contribution of about $0.3\text{--}0.5 \text{ m}^3 \text{ s}^{-1}$ of

groundwater per km was modeled (PTUA 2006). Four different homogenous river stretches of 100 m in length and $\sim 35 \text{ m}$ in section (number 35, 36, 39 and 40, Fig. 1) were investigated. They are spaced about 4–6 km, and have the typical features of a lowland river, with gentle slopes and gravel-dominated substrates. During base flow conditions, the water depth ranged from few cm to up to 60 cm.

Each site (the river stretch) was split into four different functional zones sensu BOLPAGNI et al. (2013) in order to emphasize the existence of a gradual lateral influence of groundwater resurgence across riverbed. Accordingly, it was possible to clearly identify four different functional zones, namely: i) the lateral riverbed zones directly fed by groundwater (A), ii) the river' margin (close to the banks/cliffs (B), iii) the intermediate riverbed sector (equidistant from the riverbanks and the thalweg (C), and iv) the central river sector in correspondence to the thalweg (D) (Fig. 2). Conversely, these functional zones differed only minimally in terms of sediment type. They were largely characterized by pebbles and small boulders; exclusively the functional zone A exhibited a more fine-grained sediment dominated by a muddy sand matrix. During the sampling period, the river discharge was equal to $9.4 \text{ m}^3 \cdot \text{s}^{-1}$ on average.

Algal characterization. During summer low-discharges 2013 (8–14 August), five random plots (25 × 25 cm) for each functional zone per site were sampled (for a total number of 80 plots). At each sampling plot, presence and cover-abundance values – collected using the geometrical classes (0.14, 0.28, 0.56, 1.1, 2.2, 4.4, 8.8, 18, 35, 71) provided by OKSANEN (1976) – of all visible soft-bodied benthic algal (= algal taxa including cyanobacteria attached to substrata in lotic and lentic waters, but excluding diatoms) growth form were recorded. All the different algal growth recognized in each plot were sampled, collecting a representative aliquot using tweezers, or by scraping 2–3 stones or pebbles. After an in situ rapid sorting of the sampled material, this was immediately fixed in 4% formaldehyde solution. Benthic algae were examined under a light microscope (100 to 400 or 1000 times magnification) and identified as close to species level as possible (EN 15708 2009) in agreement with several floras and monographs (e.g. KOMÁREK & ANAGNOSTIDIS 1998, 2005; ELORANTA & KWANDRANS 2007; JOHN et al. 2011). To obtain a reliable estimation of the cover-abundance values for the rare taxa 5 different slides (prepared proportionally to the cover-abundance values of the sampled algal forms) were analyzed for each sample plot. For the taxa of filamentous green algae that cannot be determined to the species level (*Spirogyra* and *Mougeotia*), categories based mainly on filament width were used (see SCHNEIDER & LINDSTRÖM 2009 for further details).

Environmental characterization. Simultaneously to algal characterization, hydrological and physico-chemical data were collected at each sampled plot using standard procedures. Water depth and velocity were recorded by a flow-probe (FP111, Global Water's); using a portable multiprobe (YSI 556 MPS, YSI, Inc., Yellow Springs, Ohio) temperature, pH, conductivity and oxygen concentration and saturation were measured. A water sample (about 250 ml) was collected by hand using a plastic bottle and filtered by GF/C filters (Whatman) and analyzed in lab for NO_3^- and soluble reactive phosphorous (PO_4^{3-}) content. NO_3^- was measured after reduction to NO_2^- by activated cadmium, and then determined spectrophotometrically using sulphanilamide and

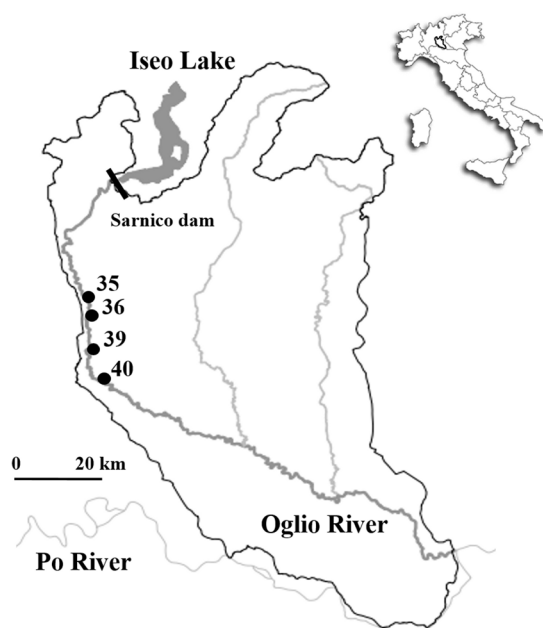


Fig. 1. Map of the Oglio River catchment with the location of sampled sites: (35) 45°27'24.14" N, 9°53'29.65" E; (36) 45°26'57.77" N, 9°53'25.90" E; (39) 45°23'57.58" N, 9°53'25.17" E, (40) 45°20'17.88" N, 9°54'14.73" E.

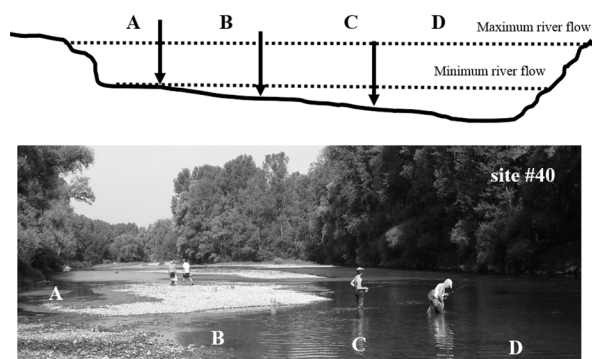


Fig. 2. Experimental design scheme: the figures illustrate the spatial distribution of the functional zones (A to D, see text for further specifications) across a sampled site (40) with respect to the water–atmosphere interface and the seasonal water–level fluctuations.

N-(1-naphtyl)ethylendiamine (detection limit = $50 \mu\text{g.l}^{-1}$) (GOLTERMAN et al. 1978). PO_4^{3-} was measured after reaction with ammonium molybdate and potassium antimonyl tartare and reduction by ascorbic acid (detection limit = $4 \mu\text{g.l}^{-1}$) (VALDERRAMA 1977). River discharges were kindly provided by the Consorzio dell'Oglio (Lombardy Region) in the frame of the project *Evaluation of the Vital Minimum Flow of the Oglio River* (2009–2015).

Statistical Analyses. A two-way ANOVA was performed to alpha-diversity (species richness) data to account for the relative effects of sites and functional zones, and for their interactions in R environment (R DEVELOPMENT CORE TEAM 2014). To see which species, if any, would indicate the different functional zones investigated we performed the IndVal analysis based on cover–abundance data using the indicpecies package for R (DE CÁCERES & LEGENDRE 2009). The statistical significance of the taxa indicator values (IV) was evaluated using a randomization procedure (999 permutations). To explore the structure in our dataset we performed an nMDS on cover–abundance data. We use the Bray–Curtis dissimilarity distance to compute the resemblance matrix between functional zones. Linear fittings were performed between the environmental variables and the output of nMDS to identify the environmental factors driving the composition of benthic algae community (function envfit, vegan package, OKSANEN et al. 2011). For each fitting we calculated the squared correlation coefficients (R^2) and p -values. In the R environment, we also tested the observed differences in species distribution patterns (based on cover–abundance values) among river' functional zones using Adonis (permutation-based multivariate analysis of variance) and Pairwise comparisons (R DEVELOPMENT CORE TEAM 2014).

RESULTS

River environment characterization

All the investigated parameters showed a clear increase or decrease pattern moving from the lateral river functional zones inwards (from A to D). Changes were more pronounced at the turn of the lateral functional zones (A and B), while minor differences were record-

ed between the inner ones (C and D) (Fig. 3).

Moving away from the functional zones A toward D, a progressive increase in water depth and velocity was recorded, passing from depths and velocities of 20 cm and 0.0 feet s^{-1} to 50 cm and 0.9 m.s^{-1} on average, respectively (Fig. 3). Similarly, temperature, pH and oxygen peaked at the functional zone D, with average values of 21°C , 7.6, and 118 %, respectively. On the contrary, conductivity and NO_3^- showed an opposite trend, with higher concentrations at the lateral zone A. Values varied in the range $405\text{--}820 \mu\text{S.cm}^{-1}$ and $3.3\text{--}12.0 \text{ mg.l}^{-1}$ (NO_3^-), respectively (Fig. 3). Compared to NO_3^- , PO_4^{3-} exhibited an opposite tendency, almost doubling its average concentration (from 4.7 to $8.2 \mu\text{g.l}^{-1}$) passing from the lateral zone A to the inner one (D).

Algal diversity, cover–abundance and distribution patterns

Over the investigated river sector, a total of 28 taxa of soft-bodied benthic algae were recorded, the majority belonged to Chlorophyceae (10 taxa), Conjugatophyceae (5), Cyanophyceae and Ulvophyceae (both 4). In addition, few taxa of Xanthophyceae (2), Rhodophyceae (1), Pheophyceae (1) and Klebsormidiophyceae (1) were recorded (Table 1).

In terms of algal diversity, moving from the marginal functional zones to the inner part of the riverbed a progressive decrease in taxa number was noted. A maximum value of 26 taxa was recorded at functional zone B (equal to the 92.9% of the local diversity noted), whereas the lowest one was recorded at the functional zone D (16 taxa, equal to the 57.1% of the local diversity noted) (Table 1, Fig. 4). Generally, no statistical differences in α -diversity were noted among sites (two-way ANOVA, $F = 2.23$, $p = 0.0932$), exclusively the functional zones (two-way ANOVA, $F = 10.32$, $p < 0.001$) and the interactions among the factors “sampling sites x functional zones” (two-way ANOVA, $F = 2.30$, $p = 0.0261$) were statistically significant.

The most widespread taxa were *Sphaerobotrys fluviatilis* BUTCHER (found at 58 sites with a percentage frequency of 72.5%), *Cladophora glomerata* (LINNAEUS) KÜTZING (49, 61.3%), *Vaucheria* cf. *aversa* HASSALL (42, 52.5%), and *Gongrosira fluminensis* F.E.FRITSCH (41, 51.3%). Cyanophyceae were scarcely represented with percentage frequencies in the range from 3.8% (*Nostoc* sp.) to 13.8% (*Lyngbya* cf. *martensiana* MENEGHINI ex GOMONT); on the contrary, the unique species recorded for both Rhodophyceae [*Hildenbrandia rivularis* (LIEBMANN) J.AGARDH] and Pheophyceae [*Heribaudiella fluviatilis* (ARESCHOUG) SVEDELIUS] shown intermediate frequency values equal to 47.5 and 43.8%, respectively.

Regarding the taxa spatial distribution, several of them exhibited clear patterns showing a preference for one or more different riverbed sectors (Table 1). *H. fluviatilis* and *H. rivularis* were concentrated at

Table 1. Algal distribution frequency (%) for all plots together (tot), and for each functional river zone separately (A to D, see text and Fig. 2 for the further details). For the *Mougeotia* and *Spirogyra* taxa that cannot be classified at the species level, we adopted a descriptive classification based on cell width (µm), number and spiraling density of chloroplasts (2K = two chloroplasts), and cell length/width ratio.

Taxon		Frequency				
		tot	A	B	C	D
Cyanophyceae						
Lyn_mar	<i>Lyngbya</i> cf. <i>martensiana</i>	13.8	5.0	5.0	30.0	15.0
Nos_sp1	<i>Nostoc</i> sp.	3.8	–	15.0	–	–
Osc_lim	<i>Oscillatoria limosa</i>	25.0	30.0	15.0	40.0	15.0
Ple_tom	<i>Plectonema tomasinianum</i>	12.5	–	5.0	15.0	30.0
Rhodophyceae						
Hil_riv	<i>Hildenbrandia rivularis</i>	47.5	75.0	100.0	15.0	–
Xanthophyceae						
Tri_vir	<i>Tribonema viridis</i>	11.3	–	20.0	25.0	–
Vau_ave	<i>Vaucheria</i> cf. <i>aversa</i>	52.5	10.0	30.0	75.0	95.0
Phaeophyceae						
Her_flu	<i>Heribaudiella fluviatilis</i>	43.8	55.0	80.0	35.0	5.0
Klebsormidiophyceae						
Kle_sp	<i>Klebsormidium</i> sp.	2.5	–	10.0	–	–
Chlorophyceae						
Chae_inc	<i>Chaetophora incrassata</i>	13.8	5.0	15.0	35.0	–
Dra_glo	<i>Draparnaldia glomerata</i>	2.5	10.0	–	–	–
Gon_flu	<i>Gongrosira fluminensis</i>	51.3	40.0	70.0	55.0	40.0
Hyd_ret	<i>Hydrodictyon reticulatum</i>	37.5	40.0	45.0	55.0	10.0
Mic_abb	<i>Microspora abbreviata</i>	10.0	30.0	10.0	–	–
Oed_cal	<i>Oedogonium calcareum</i>	8.8	10.0	25.0	–	–
Oed_cap	<i>Oedogonium capillare</i>	32.5	25.0	75.0	15.0	15.0
Sph_flu	<i>Sphaerobotrys fluviatilis</i>	72.5	55.0	70.0	95.0	70.0
Sti_ten	<i>Stigeoclonium</i> cf. <i>tenuis</i>	6.3	–	–	–	25.0
Tet_jav	<i>Tetrasporidium javanicum</i>	16.3	10.0	15.0	20.0	20.0
Ulvophyceae						
Cla_glo	<i>Cladophora glomerata</i>	61.3	15.0	30.0	100.0	100.0
Rhi_hie	<i>Rhizoclonium hieroglyphicum</i>	43.8	5.0	25.0	55.0	90.0
Ulo_aeq	<i>Ulothrix aequalis</i>	11.3	30.0	15.0	–	–
Ulo_zon	<i>Ulothrix zonata</i>	18.8	30.0	30.0	15.0	–

Table 1 Cont.

Conjugatophyceae						
Mou_sp1	<i>Mougeotia</i> sp. (14–20 µm)	10.0	30.0	10.0	–	–
Spi_sp1	<i>Spirogyra</i> sp1 (28–35 µm, 2K, l/b>2)	20.0	20.0	20.0	25.0	15.0
Spi_com	<i>Spirogyra communis</i>	32.5	20.0	50.0	40.0	20.0
Spi_neg	<i>Spirogyra neglecta</i>	31.3	–	30.0	55.0	40.0
Zyg_ste	<i>Zygnema</i> cf. <i>stellinum</i>	12.5	35.0	15.0	–	–
α-diversity		28	22	26	19	16

the functional zone B, with percentage frequencies of 80.0% and 100.0%, respectively. A similarly result was recorded for *G. fluminensis* (with a percentage of 70.0%). Conversely, the presence of *C. glomerata*, *V. cf. aversa*, and *Rhizoclonium hieroglyphicum* (C.AGARDH) KÜTZING peaked at the functional zones C and D, with rates equal to 100%, 75.0–95.0%, and 55.0–90.0%, respectively (Table 1).

Cover–abundance values confirmed and better delineated the spatial patterns of taxa suggested by the distribution frequencies of algae (Tables 1, 2). Exclusively few taxa showed mean cover–abundance values higher than 0.5 (4 out 28 at the global scale, and 11 out 28 considering the four functional zones separately) (Table 2). Generally, the cover–abundance rates were low, not exceeding values of about 18; exclusively at the central sectors of the riverbed algae showed average values in the range 30.5–35.3. The most representative species was *C. glomerata*, with a mean cover–abundance value of 11.9, followed by *V. cf. aversa* with a value of 3.9. *C. glomerata* dominated the functional zones D and C (in co–dominance with *V. cf. aversa*), *Hydrodictyon reticulatum* (LINNAEUS) BORY dominated the functional zone B (in co–dominance with *H. rivularis*), and finally *H. rivularis* dominated the functional zone A (in co–dominance with *H. fluviatilis*) (Table 2). All these evidences were further confirmed by the IndVal analysis. A total of 15 indicator species were identified (Table 3). Focusing on the most significant ($p < 0.001$), *Zygnema* cf. *stellinum* (O.F.MÜLLER) C.AGARDH (IV = 0.56) was identified as indicator species for the functional zone A, *Oedogonium capillare* KÜTZING ex HIRN (IV = 0.73) and *H. rivularis* (IV = 0.72) for B, *V. cf. aversa* (IV = 0.73) for C, and *C. glomerata* (IV = 0.81) and *R. hieroglyphicum* (IV = 0.70) for the zone D (Table 3).

The ordination maps obtained by nMDS analysis confirmed the existence of a clear segregation of taxa among functional zones, with the exception of zones A and B that are partially overlapped one another (Fig. 5). Exploring the correlation between nMDS outputs and environmental variables, all the variables considered showed statistically significant square coef-

ficients. However, the most important variables were: pH ($R^2 = 0.82$, $p < 0.001$), NO_3^- ($R^2 = 0.78$, $p < 0.001$), flow velocity ($R^2 = 0.76$, $p < 0.001$), conductivity ($R^2 = 0.74$, $p < 0.001$), water depth ($R^2 = 0.70$, $p < 0.001$), and PO_4^{3-} ($R^2 = 0.68$, $p < 0.001$). Oxygen and temperature displayed squared coefficients equal to 0.48 with a p -value of 0.015. The Adonis results further underlined the marked differentiation between sites ($p < 0.001$) and functional zones ($p < 0.001$) (Table 4), emphasizing the predominant role of the functional zones (i.e. groundwater resurgence) which alone represented more than 38% of the explained variance. The Pairwise comparisons substantiated the existence of significant differences ($p < 0.02$) among all the functional zones combinations with the exception of the functional zone A in comparison to B ($p = 0.552$). The total algal diversity remained constant among sites (as verified by two-way ANOVA analysis); however, the Adonis test suggested a rapid turnover in algal taxa among sites, as well as the existence of diversified algal communities in terms of cover–abundance values or species dominance.

DISCUSSION

Summarizing, along the investigated river reach a clear segregation both for taxa and for environmental data was observed, especially in terms of relative taxa presence and cover–abundance, pH, hydrological variables and nutrient availability. Overall, we observed an important contribution of groundwater supply in shaping the structure and complexity of algal communities at the microhabitat scale (in the meter range) during the summer river base flow. *C. glomerata*, *R. hieroglyphicum* and *V. cf. aversa* dominated the river's central zones (D + C) and were indicators of rather high pH values (> 7.2) and flow velocities of about 1.5 m.s^{-1} , and low NO_3^- content (3.7 mg.l^{-1} in average). On the contrary, the marginal functional zones (B + A) exhibited a more diversified algal communities with the local dominance of *O. capillare*, *H. rivularis* and *Z. cf.*

Table 2. Algal cover–abundance mean values – based on the geometric classes provided by OKSANEN 1976 – for all plots together (tot, $n = 80$), and for each functional river zone separately (A to D, see text for the further details; $n = 20$); the number of colonized plots was reported in brackets. Only taxa with values > 0.5 are shown; for the taxa abbreviations see Table 1.

Taxon	Cover–abundance mean values ($n =$ colonized plot)				
	tot	A	B	C	D
Rhodophyceae					
Hil_riv	0.8 (38)	1.6 (15)	1.7 (20)	–	–
Xanthophyceae					
Vau_ave	3.9 (42)	–	–	10.9 (15)	4.1 (19)
Phaeophyceae					
Her_flu	0.6 (33)	1.1 (11)	1.1 (14)	–	–
Chlorophyceae					
Hyd_ret	1.3 (30)	1.2 (8)	2.0 (9)	1.9 (11)	–
Mic_abb	–	0.6 (6)	–	–	–
Oed_cap	–	–	0.7 (15)	–	–
Sph_flu	1.2 (58)	0.7 (11)	1.9 (14)	1.6 (19)	–
Ulvophyceae					
Cla_glo	11.9 (49)	–	–	16.2 (20)	31.2 (20)
Ulo_aeq	–	0.8 (7)	–	–	–
Conjugatophyceae					
Spi_com	–	–	0.6 (10)	–	–
Zyg_ste	–	0.5 (6)	–	–	–

stellinum which were seen to act as indicators of rather high NO_3^- content (up to 11.7 mg.l^{-1}), low flow velocities ($< 0.5 \text{ m.s}^{-1}$) and pH values (in the range 6.4–7.6). Indeed, pH, NO_3^- , water velocity and conductivity were the most important drivers in explaining the observed algal spatial patterns.

All these evidence are in line with previous works, highlighting the pivotal role played by the

Table 3. Indicator species for the four functional zones investigated (A to B; see text for further specifications); for the taxa abbreviations see Table 1. The statistical significance (P) of the indicator values (IVs) was tested using Monte Carlo permutation tests.

Functional zone	IV	P	
A			
Zyg_ste	0.56	0.0008	***
Ulo_aeq	0.53	0.0027	**
Mou_sp1	0.52	0.0032	**
Mic_sp.	0.47	0.0090	**
Ulo_zon	0.46	0.0499	*
B			
Oed_cap.	0.73	0.0001	***
Hil_riv	0.72	0.0001	***
Spi_com	0.59	0.0052	**
Her_flu	0.59	0.0047	**
Oed_cal	0.44	0.0286	*
C			
Vau_ave	0.73	0.0006	***
Chae_inc	0.51	0.0058	**
D			
Cla_glo	0.81	0.0001	***
Rhi_hie	0.70	0.0001	***
Sti_ten	0.50	0.0023	**

physico–chemical determinants in shaping river benthic algal assemblages, but not confirm a lesser importance of NO_3^- and conductivity than P (SCHENIDER et al. 2013; ROTT & SCHENIDER 2014). This is probably due to the relative diffuse nitrate contamination of groundwater and the importance of aquifer resurgence in fed surficial water bodies in the studied area. The locally high NO_3^- contamination makes difficult to explain the natural groundwater NO_3^- content, as highlighted by LIMBRICK (2003) in the south Dorset (UK), and can blind the importance of the other ecological determinants in driving algal assemblages. However, in total agreement with those by GARCÍA & ABOAL (2014) for the Pego–Oliva marsh (East Iberian Peninsula), our data confirm the presence of well–diversified soft–bodied benthic algal communities under unusually high nitrogen concentrations. This suggests the probably existence of adaptation processes in the establishment and development of algae in heavily impacted floodplains. Similar evidences were recorded by BOLPAGNI et al. (2015) investigating the spatial distribution of *Thorea hispida* (THORE) DESVAUX along the Oglio River. Another important element can be traced in part to the

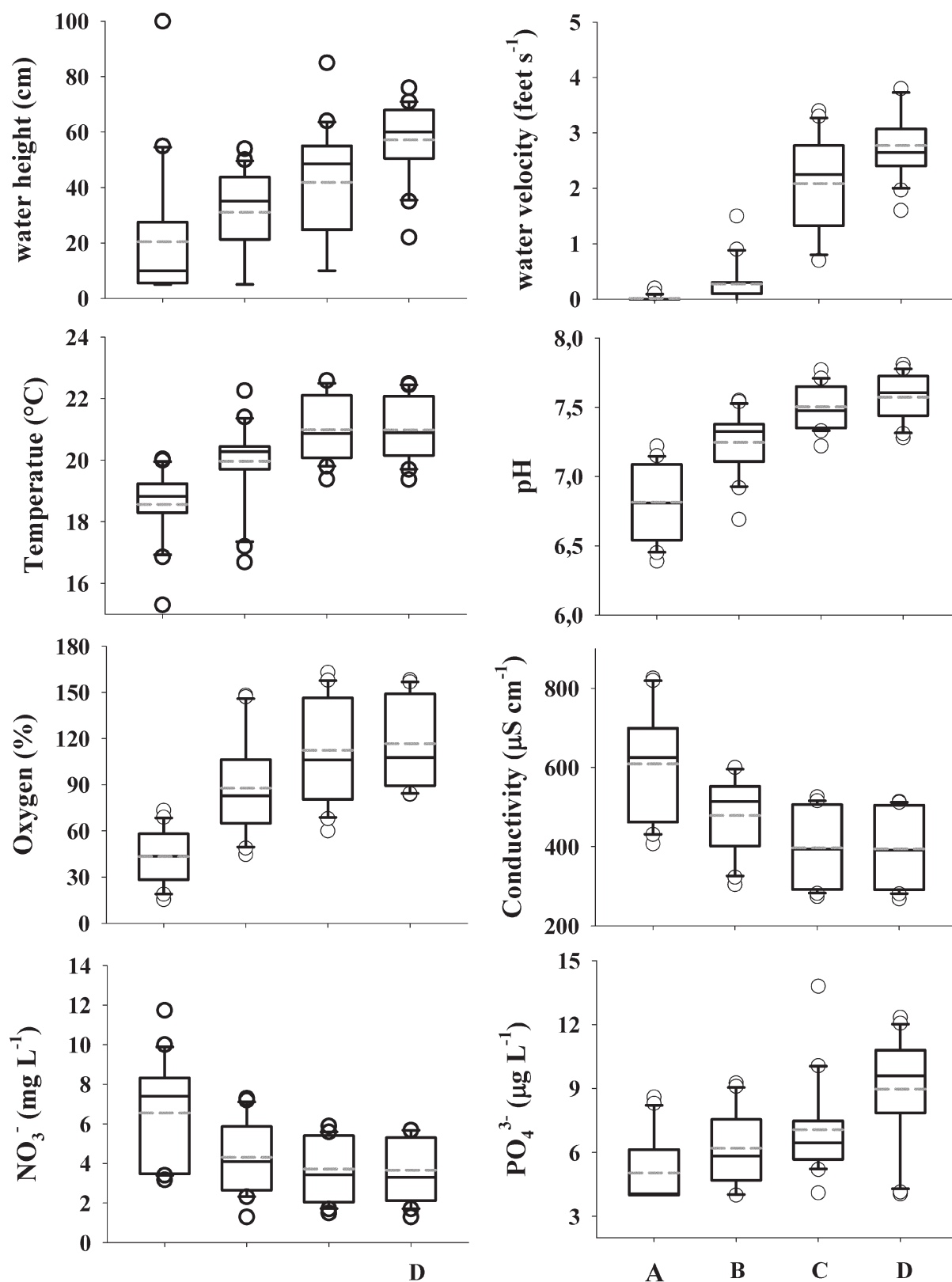


Fig. 3. Box-whiskers plots (minimum, 25%, median, mean = grey dashed line, 75%, maximum, and outliers = dots) showing variability in water depth, water velocity, temperature, pH, oxygen, conductivity, NO₃⁻ and PO₄³⁻ concentrations at the four different river functional zones investigated (A, B, C, and D); see text for further specifications.

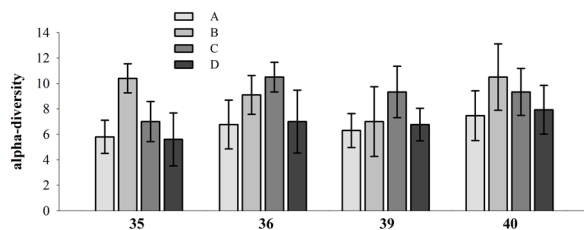


Fig. 4. Algal diversity (mean \pm standard deviation; $n = 5$) at the functional zone scale (A to D; see text for further specifications).

Table 4. The results of the Adonis analysis used to test the direct effects of sites (S) and functional zones (FZ) on the taxa composition of algal communities [(Df) degrees of freedom; (SS) sum of squares; (Ev) explained variance (%); (MS) mean squares; (F) F-statistic; (p) p-value based on permutations].

	Df	SS	MS	Ev	F	p
S	3	2.47	0.82	9.5	6.76	<0.0001
FZ	3	10.35	3.45	39.8	28.37	<0.0001
St x FZ	9	5.41	0.60	20.8	4.94	<0.0001
Residuals	64	7.78	0.12		0.30	
Total	79	26.00	1			

riverbed microhabitat complexity. As stressed by KRUPPEK et al. (2012) sampling different streams in the Brazilian subtropical region, the macroalgal community structure seems to be strictly driven by the microhabitat variables, especially in term of substrate type. However, in our system, moving from the functional zone A to D, the substrate changed only moderately from a predominantly muddy–sandy character with small pebbles (A) to an hard one co-dominated by pebbles and small boulders (B to D). Consequently, we do not believe that the gradient in substrate structure can justify the significant differences observed in terms of algal diversity and cover–abundance. In this context, the influence of groundwater supply in supporting algal diversity should be further investigated. Indeed, KUGLEROVÁ et al. (2014) considering the riparian river sectors of rivers largely fed by groundwater demonstrated the strong positive role of water resurgence on local plant diversity. The increase in soil pH as well as of the nitrogen availability (manifested as lower soil C/N ratio) associated to the aquifer resurgence were considered the main drivers of this species number increase. Present data support this evidence and stress the importance of groundwater supply in the spatial patterns of riverine primary producers.

Our data support also the not-negligible contribution of the hydrological features (flow velocity and water depth) in driving the spatial arrangement of algae across a riverbed, as widely expected for other fundamental river biological communities (GUARESCHI et

al. 2014). These factors are normally neglected in the field surveys and monitoring programs based on algae. However, several evidences have suggested the central role of water flow dynamics on algal establishment and development, especially in impacted and regulated watercourses (VILLENEUVE et al. 2011). For example, investigating the spatial patterns of filamentous green algae in a large fluvial lake (Lake Saint–Pierre, Canada), CATTENEO et al. (2013) verified the predominance of the hydrological variables as predictors of algae occurrence and no effect of water quality. Similarly, ANDRUS et al. (2015) verified the pivotal contribution of both water flow and velocity in influencing the assemblage structure of algae in agricultural streams (Midwest, USA). Many authors have also stressed the contributions (both positive and negative) of fluctuations in current velocity in determining the characteristics of fixed algae communities. These effects may be summarized in an increase in nutrient delivery and a greater oxygenation of water, or in the abrasive mechanical effects of water flow (BRANCO & NECCHI JÚNIOR 1997). With respect to the potential filtering effect of shading on algae diversity and their spatial distribution (STEINMAN & MCINTIRE 1987), along the Oglio River reach analysed it could be considered marginal. Exclusively at the functional zone A the shading could reach values of 20%; in general, shading values constantly in the range 0 to 5% were recorded.

All these reflections acquire enhanced emphasis considering the central role assigned to the macro-algae in the monitoring programs of river health. Hence, based on the evaluation reported by HAURY et al. (2006), among the species recorded along the Oglio River, some can be considered quite good indicators of well-oxygenated and clear waters. *H. rivularis* and the genera *Mougeotia*, *Tetraspora* and *Zygnema* show a water quality score (Csi) higher than 12 in a scale of 0 (heavy organic pollution and heterotrophic taxa) to 20 (oligotrophy). These evaluations contrast in part with the current physico-chemical quality status of the Oglio River as well as the N surplus generated in its catchment (SOANA et al. 2011). A possible explanation is that these taxa, generally found in the lateral functional zones of the sites investigated, seem to be more driven by the temperature or the imbalance in the C:N:P ratio than the chemical determinants availability per se. In fact, the above-mentioned taxa are not typical of lowland rivers but of low order watercourses (streams) in mountain regions, especially *H. rivularis* (SHEATH & HAMBROOK 1990). ABDELAHAD et al. (2015) recorded similar results by surveying a large set of lowland freshwater springs located in a narrowed heavily impacted floodplain area (30 \times 12 km), largely placed within the Oglio River basin. They revealed a marked difference in the physical and chemical preferences of Batrachospermales applying a niche analysis approach, suggesting a major role for NO_3^- in the appearance of morphological deformities in the red algal

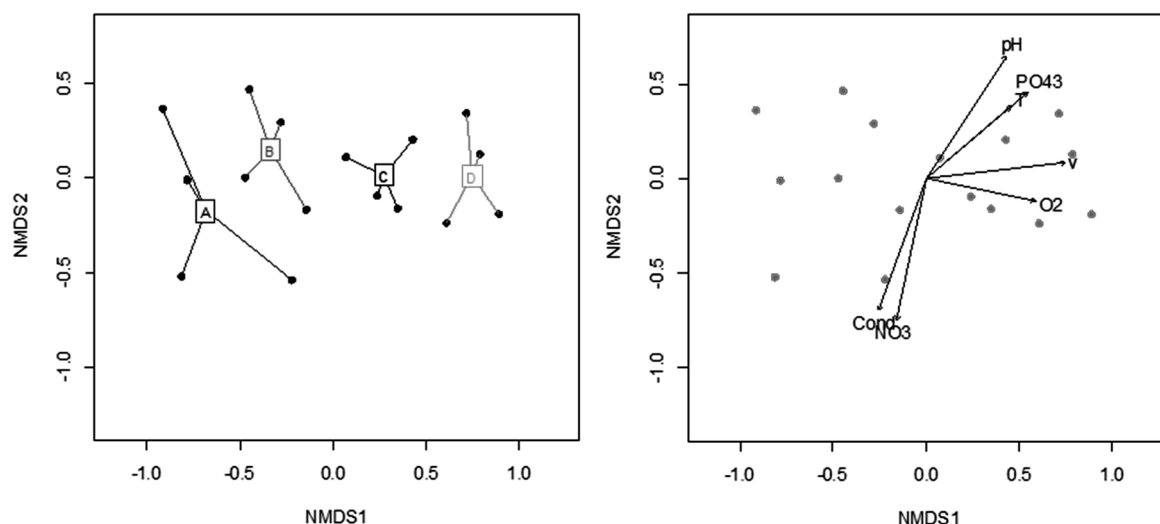


Fig. 5. Non-metric multidimensional scaling (NMDS) ordination of algal communities in a two-dimensional space. The left plot combines individual sample plots with the average score of each functional zone (A to D; see text for further specifications). In the right plot, the environmental vectors have been fitted onto the ordination diagram (see Table 4 for abbreviation and details).

reproductive structures. Furthermore, the widespread occurrence of red algae in presence of rather cold and impacted waters, with NO_3^- concentrations in the range $19.1\text{--}53.6 \text{ mg.l}^{-1}$, was mainly associated by the stoichiometric imbalance among nutrients. Locally, in the emergent groundwater the dissolved reactive phosphorus was completely absent due to the trapping by the soil-matrix groundwater system.

The present data integrate previous works on the importance of spatial variables in shaping benthic algal communities (TANG et al. 2013), introducing a non-negligible role of micro-scale features of riverbeds in supporting the patchiness of algal mats. In a few meters, we recorded a clear succession of algal communities in presence of wide variations in terms of hydrological and physico-chemical determinants (such as pH, NO_3^- and conductivity). However, at the site scale the two-way ANOVA and Adonis analyses have shown contrasting results, suggesting the existence of multiple forces and complex interactions in shaping the algal communities (both in terms of species diversity and abundance). A possible explanation may be the rapid variation (in space) in the magnitude of groundwater supply (in terms of NO_3^- availability) along the river sector in analysis.

All these considerations shed also new light on the response of algal taxa to a relevant groundwater ingress in a lowland river. This phenomenon is capable of creating steep gradients along the lateral spatial dimension of the river and can profoundly influence the multidimensional interactions between the river channel and its catchment. Furthermore, our data suggest the need for an appropriate definition of spatial scales in order to a correct assessment of the trophic requirements of algal taxa as stressed by TANG et al. (2013). We would like to reiterate the need to join regional studies (i.e. macro-scale investigations) with

depth analyses on the micro-spatial gradients. Only in this way, it will be possible obtain a proper ecological assessment of individual species taking into account their adaptability to changes in the trophic conditions of water bodies colonized.

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