Disentangling the multiple stressors acting on stream ecosystems to support restoration priorities

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INTRODUCTION

Stream ecosystems may suffer from the effects of multiple stressors. Planning restoration actions without knowing the relative weight of each stressor might lead to disproportionately costly or ecologically meaningless measures. This is particularly relevant under the EU Water Framework Directive (WFD) (European Commission 2000) where economic considerations play a role in justifying exemp-tions from the overarching aim of the directive of achieving the good ecological status in all the EU water bodies by 2015. According to the WFD, if reaching the good ecological status proves to be disproportionately costly, either the 2015 deadline may be extended, or the objective be relaxed. Following the same rationale, the WFD requires a distinction be made between 'natural' and 'heavily modified water bodies' (HMWBs). HMWBs may have an acceptably lower ecological status as the result of hydromorphological pressures, which cannot be removed because of the high economic cost. In agreement with the WFD prescriptions, Italy has adopted new water quality standards. Besides the environmental quality stan-dards set by Directive 2008/105/EC (European Commission 2008), other chemical standards have been adopted by the Italian law concerning some macropollutants, in support for the good ecological status. These new macropollutant standards are aggregated into an index (i.e. LIMeco according to the legislative decree n.152, 2006) which considers dissolved oxygen, ammonia and nitrate concentration, and total phosphorus. To each of these parameters, a score is attributed according to the thresholds shown in Table 1, and based on the average of the four parameter scores the water quality is classified. LIMeco index classification is very restrictive, particularly concerning the parameters nitrate and phosphorus, making extremely difficult if not challenging the achievement of water quality objectives for many Italian rivers. In a previous study Azzellino *et al.* (2013) showed that the achievement of the 'good quality status' according to the LIMeco index, in an urban effluent-dominated watershed, would be technically feasible but paradoxically end in damaging the river ecology due to the lack of selectivity of the needed treatments.

In this study, the ecological quality status of several river systems is correlated with the available proxies of the multiple stressors affecting the systems, providing elements in support of the restoration scenarios that may maximize the effectiveness/cost ratio.

Table 1 | LIMeco index recently enforced by the Italian legislation

	Thresholds					Classification based on LIMeco scores	
LIMeco	**High**	**Good**	**Moderate**	**Poor**	**Bad**		
100-DOsat[a]	≤ 10	≤ 20	≤ 40	≤ 80	>80	≥ 0.66	High
N-NH_4 (mg/l)	<0.03	≤ 0.06	≤ 0.12	≤ 0.24	>0.24	≥ 0.50	Good
N-NO_3 (mg/l)	<0.6	≤ 1.2	≤ 2.4	≤ 4.8	>4.8	≥ 0.33	Moderate
Total-P (μg/l)	<50	≤ 100	≤ 200	≤ 400	>400	≥ 0.17	Poor
						<0.17	Bad
Score	1	0.5	0.25	0.125	0		

[a] DOsat is dissolved oxygen at saturation.

Scores need to be assigned according to the thresholds, and the final score is the average of the four parameter scores.

MATERIAL AND METHODS

Study area

The Lombardy region is part of the Po river district, the longest Italian river, with a watershed area of about 71,000 km^2 (almost a quarter of the national surface area), a stream length of 652 km, 141 tributaries and a delta surface area of 380 km^2 (see Figure 1). Lombardy is characterized by high population densities and elevated agricultural productivity, one of the highest of the whole of Europe.

Used data

All the measurements used here come from the monthly monitoring activity, carried out by ARPA, the Italian Regional Environmental Protection Agency, during the period 2009–2011 at 120 sampling stations in the Lombardy region (see Table 2). Such water quality monitoring refers mainly to low or mean-flow conditions; less than 25% of the measurements available concern higher flow conditions. The analysis was focused on the annual averaged statistics of every station in order to

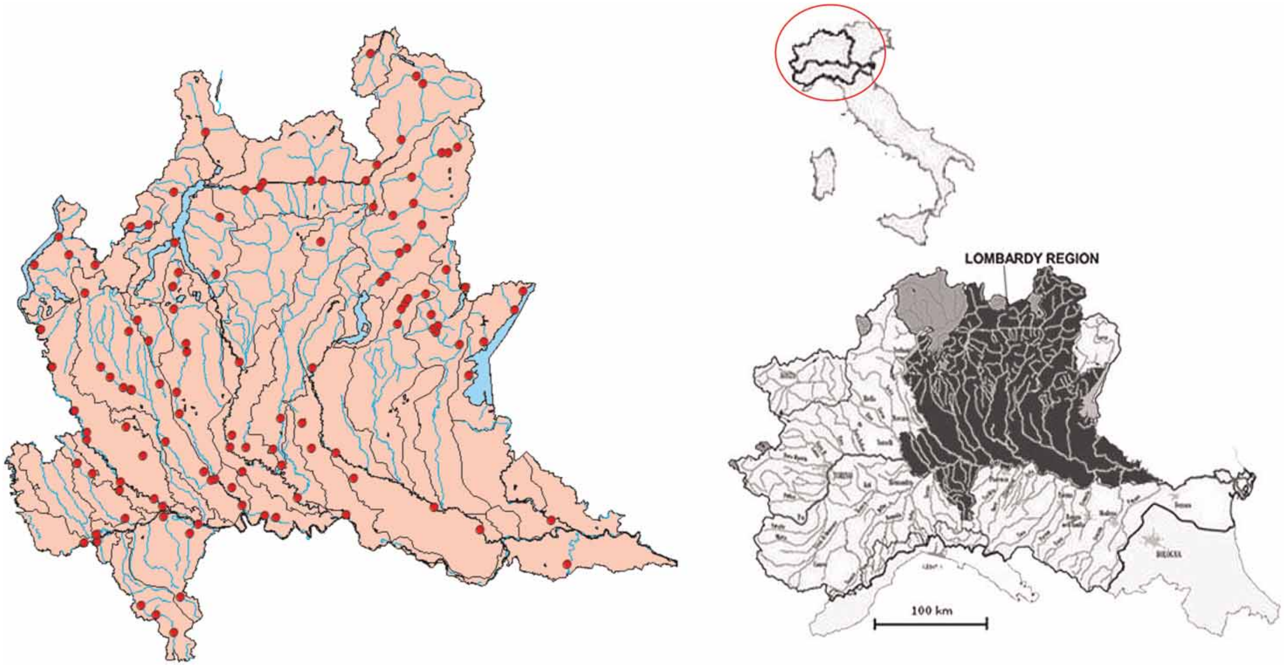

Figure 1 | Study area: the Lombardy region is shown within the Po River watershed (map on the right). The 120 monitoring stations used in this study are also shown (dots in the map on the left).

Table 2 | Annual averaged statistics of the water quality parameters considered for the studied period (2009–2011)

	N	Mean	Median	Minimum	Maximum	Std deviation
BOD (mg/l)	99	2.54	2.33	1.00	6.75	1.02
COD (mg/l)	99	7.94	5.87	2.00	34.75	5.53
Conductivity (μS/cm)	86	302.33	279.42	39.50	980.75	209.74
Hardness (mg/l)	99	160.66	153.83	17.50	500.33	99.82
SS (mg/l)	99	22.41	5.50	1.00	1,249.50	125.68
Water temperature (°C)	99	12.86	13.25	5.85	19.86	3.52
pH	99	7.97	7.98	7.28	8.60	0.28
P-PO_4 (mg/l)	99	0.10	0.03	0.00	0.69	0.16
N-NO_3 (mg/l)	99	1.67	1.13	0.12	7.15	1.49
S-SO_4 (mg/l)	99	34.34	26.83	2.00	195.50	36.23
Cl (mg/l)	99	12.13	4.70	0.49	121.80	19.83
Total-N (mg/l)	99	2.48	1.45	0.20	14.00	2.49
N-NH_3 (mg/l)	45	0.27	0.09	0.01	1.38	0.38
N-NH_4 (mg/l)	76	0.14	0.08	0.02	2.09	0.29
Total-P (mg/l)	51	0.10	0.05	0.01	1.17	0.20
Percent DOsat (%)	92	96.22	97.76	71.25	109.52	6.85
DO (mg/l)	99	9.95	10.08	6.70	12.66	0.96
Escherichia coli (UFC/100 ml)	90	7,256	1,419	9	110,000	17,341
Streamflow (m^3/s)	61	2.42	1.18	0.08	28.05	4.29
LIMeco	120	0.59	0.62	0.10	1.00	0.24
STAR_ICMi	120	0.63	0.65	0.10	1.13	0.25
Diatoms	120	0.78	0.77	0.32	1.38	0.20
Macrophytes	16	0.72	0.72	0.43	0.99	0.14

BOD: biochemical oxygen demand; COD: chemical oxygen demand; SS: suspended solids; DO: dissolved oxygen.

maximize the robustness of the correlation analysis and to minimize the influence of outliers. Data about macroinvertebrate assemblages were also collected in the same period and the STAR Intercalibration Common Metric Index (Star_ICMi, Erba *et al.* (2009)), now broadly used in Europe to enforce the WFD, was determined. Macroinvertebrates were sampled quantitatively following a multihabitat scheme according to the AQEM and STAR sampling protocols (AQEM after Hering *et al.* (2004) and Furse *et al.* (2006)). For each sampling campaign in each site, a total of 10 replicates covering 0.1 m^2 each, using surber nets (500 μm mesh) were collected. Each sample of macroinvertebrates was sorted and partially identified in the field, kept in 70% ethanol and transported to the laboratory for further taxonomical identification. All specimens have been identified at family level. Sampling campaigns have been performed seasonally (four times in each year), but we retained for the subsequent analyses only data that could be matched with water quality and hydrological data of the same period. Moreover, diatoms and macrophytes – the latter at a very limited number of stations – were also sampled. Diatoms were sampled from natural substrata such as pebbles, cobbles and boulders with a combined exposed surface area of *ca.* 100 cm^2. The upper part of the stone substratum was scrubbed with a toothbrush as recommended by Kelly *et al.* (1998).

Diatom sampling, sample treatment, and laboratory work were carried out according to the European recommendations (EN 13946 2003 and EN 14407 2004) and national guidelines (ISPRA-APAT 2008) and a multimetrics index (ICM_d) was calculated also for diatoms according to Mancini & Sollazzo (2009). Finally, Corine land cover data (2000) and wastewater regional statistics (i.e. people equivalents and wastewater volumes) were used as proxies of anthropic stressors and correlated with water quality profiles. Particularly land uses were evaluated in terms of density (e.g. arable area/watershed area).

Statistical methods

Factor analysis (FA) was performed based on the correlation matrix of the measurements (according to Afifi & Clark (1996)). FA was obtained through a preliminary principal component analysis (PCA) which extracted the eigenvalues and eigenvectors from the covariance matrix of the original variances. FA was chosen to reduce the contribution of the less significant parameters within each component, by extracting a new set of varifactors through rotating the axes defined by the PCA extraction. The varimax rotation criterion was used to rotate the PCA axes allowing their orthogonality to be maintained. The number of factors to be retained was chosen on the basis of the 'eigenvalue higher than 1' criterion (i.e. all the factors explaining less than the variance of a single standardized variable were discarded). That allowed the selection of a few factors able to describe the whole data set with minimum loss of original information. FA allowed elimination of the uninformative variables, maintaining only the most significant if multicollinearity occurs. A hierarchical cluster analysis (CA) was used to analyze the similarities among the water quality profiles using the Euclidean distance as distance metric (Equation (1)).

$$d_2(x_i, x_j) = \sqrt[2]{\sum_{k=1}^{q} (x_{ik} - x_{jk})^2} \tag{1}$$

where i and j refer to a couple of stations, and k to the considered parameters.

CA was run based on the FA varifactors and Ward's method was used as the cluster method. Finally, quantile and multiple linear regression methods were used to correlate the STAR_ICMi 90th percentile values to the corresponding chemical ranges. The statistical packages SPSS ver. 21.0 and GRETL (Gnu Regression, Econometrics and Time-series Library, Cottrell & Lucchetti (2011)) were used to perform all the statistical analysis.

RESULTS AND DISCUSSION

PCA/FA extracted eight components (*F*1–*F*8), explaining 83% of the total variance (see Table 3). As can be observed the first component after the PCA extraction was by far the most informative, alone explaining 35% of the variance. After rotation the total variance of the first component is reduced to 22% and the variance explained by varifactors 2 and 3, 4 and 5 is very similar. Table 4 gives the factor loadings matrix to the rotated varifactors showing the most meaningful parameters within each varifactor. Parameters that lie on the same component are reasonably correlated. Correlated parameters may possibly derive from the same source, e.g. conductivity and hardness both lying on varifactor 3. As shown by the factor loadings, the eight varifactors have distinct characteristics:

F1 includes parameters which are typical of the treated wastewaters such as COD, P-PO_4, N-NO_3; it correlates with the wastewater volume/river volume ratio and is inversely correlated with LIMeco index;

F2 correlates with parameters typical of the untreated wastewaters such as biochemical oxygen demand (BOD), N-NH_4 and *Escherichia coli*;

F3 correlates with parameters typical of the mineral composition of the rocks associated with the water (e.g. conductivity, hardness, sulfates);

Table 3 | Total explained variance after PCA/FA extraction

	Extraction sums of squared loadings			Rotation sums of squared loadings		
Varifactor	**Total variance**	**% Variance**	**Cumulative % variance**	**Total variance**	**% Variance**	**Cumulative % variance**
1	9.14	35.15	35.15	5.86	22.53	22.53
2	2.77	10.65	45.80	2.92	11.24	33.77
3	2.28	8.78	54.58	2.91	11.20	44.96
4	2.11	8.13	62.71	2.34	9.00	53.96
5	1.72	6.62	69.34	2.32	8.93	62.89
6	1.29	4.96	74.29	2.22	8.53	71.43
7	1.27	4.88	79.18	1.81	6.96	78.38
8	1.05	4.05	83.23	1.26	4.84	83.23

Both the unrotated (i.e. PCA on the left) and the rotated (i.e. FA on the right) solutions are shown.

Table 4 | Factor loadings to the rotated varifactors

	Varifactors							
Rotated component matrix	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
LIMeco	− **0.610**	−0.159	−0.235	−0.228	0.217	−0.405	−0.075	−0.139
Population density (inhab/m^2)	0.318	0.347	−0.037	0.067	−0.093	0.057	**0.811**	0.022
Cultivated land density (arable area/total area)	−0.062	−0.257	−0.132	0.054	**0.605**	0.021	0.428	−0.063
Forest and wood density (forest area/total area)	−0.030	0.070	−0.046	−0.101	**0.909**	−0.033	−0.124	0.094
Forest and cultivated land density ((arable + forest)/ total area)	−0.011	0.011	−0.006	−0.031	**0.959**	0.002	−0.030	0.037
Urban density (urban area/total area)	−0.060	−0.044	0.002	−0.003	0.013	−0.059	**0.939**	−0.010
Cumulative people equivalent	0.073	−0.002	0.019	0.062	−0.008	**0.984**	−0.014	−0.018
Wastewater volume (m^3/s)	0.082	0.025	−0.022	0.049	0.005	**0.983**	−0.018	−0.010
Wastewater volume/river volume	**0.758**	−0.031	−0.081	−0.105	0.010	0.296	0.103	0.041
BOD (mg/l)	0.466	**0.634**	−0.055	−0.174	0.038	0.030	−0.033	0.149
COD (mg/l)	**0.776**	0.313	0.235	0.268	0.023	0.005	0.010	−0.121
Conductivity (μS/cm)	0.397	0.086	**0.835**	0.224	−0.095	−0.021	0.033	0.058
Hardness (mg/l)	0.257	−0.021	**0.892**	0.054	−0.104	0.015	−0.025	0.189
Suspended solids (mg/l)	−0.032	−0.022	0.032	−0.124	−0.046	0.014	0.000	− **0.812**
Temperature (°C)	0.346	−0.103	0.170	**0.677**	−0.328	0.058	0.160	0.185
pH	−0.157	0.006	**0.533**	−0.193	0.078	0.006	−0.017	**0.628**
P-PO$_4$ (mgP/l)	**0.831**	0.453	0.041	0.208	−0.015	0.026	0.062	−0.017
N-NO$_3$ (mgN/l)	**0.815**	0.095	0.294	0.242	−0.086	−0.020	0.010	0.069
SO$_4^-$ (mg/l)	0.079	0.084	**0.849**	−0.053	−0.005	0.016	−0.045	−0.123
Cl$^-$ (mg/l)	**0.808**	0.177	0.303	0.269	−0.016	−0.032	−0.014	−0.121
Total-N (mg/l)	**0.829**	0.316	0.190	0.289	−0.048	−0.010	0.023	0.001
N-NH$_4$ (mg/l)	0.209	**0.787**	0.156	0.259	−0.024	−0.038	0.039	−0.065
DOsat (%)	−0.296	−0.441	0.001	− **0.691**	−0.052	−0.089	−0.009	0.108
DO (mgO$_2$/l)	−0.342	−0.263	0.021	− **0.832**	−0.001	−0.080	0.037	−0.055
E. coli (UFC/100 ml)	0.279	**0.795**	0.034	0.188	−0.045	0.054	0.111	0.039
Total-P (mg/l)	**0.758**	**0.559**	0.035	0.228	0.009	0.003	0.051	−0.063

Loadings higher than 0.5 and lower than −0.5 are in bold.

F4 correlates with dissolved oxygen;

F5 correlates with the presence of arable lands and woodlands;

F6 correlates with wastewaters in terms of volume and of people equivalent (i.e. 60 g BOD per day per inhabitant);

F7 correlates with population density and the presence of urban areas;

F8 correlates with suspended solids (SS) and pH.

It is worthwhile to remark that the LIMeco index correlates with pollutants typical of the treated wastewater, since ammonia and dissolved oxygen, although both present in the LIMeco index, lie on different varifactors. Based on these varifactors the hierarchical CA was performed and six clusters were identified. Figure 2 shows the characteristics of these clusters in terms of standardized varifactors (i.e. with mean equal to 0 and standard deviation equal to 1):

cluster 1 (*n*: 56) has average characteristics with the exception of temperature higher than the average and the low presence of untreated wastewaters; this is the typical profile of lowland stations;

cluster 2 (*n*: 7) has most of the pollutant varifactors lower than the average and a much higher presence of green land use (i.e. arable land and woods); this is the typical profile of alpine stations;

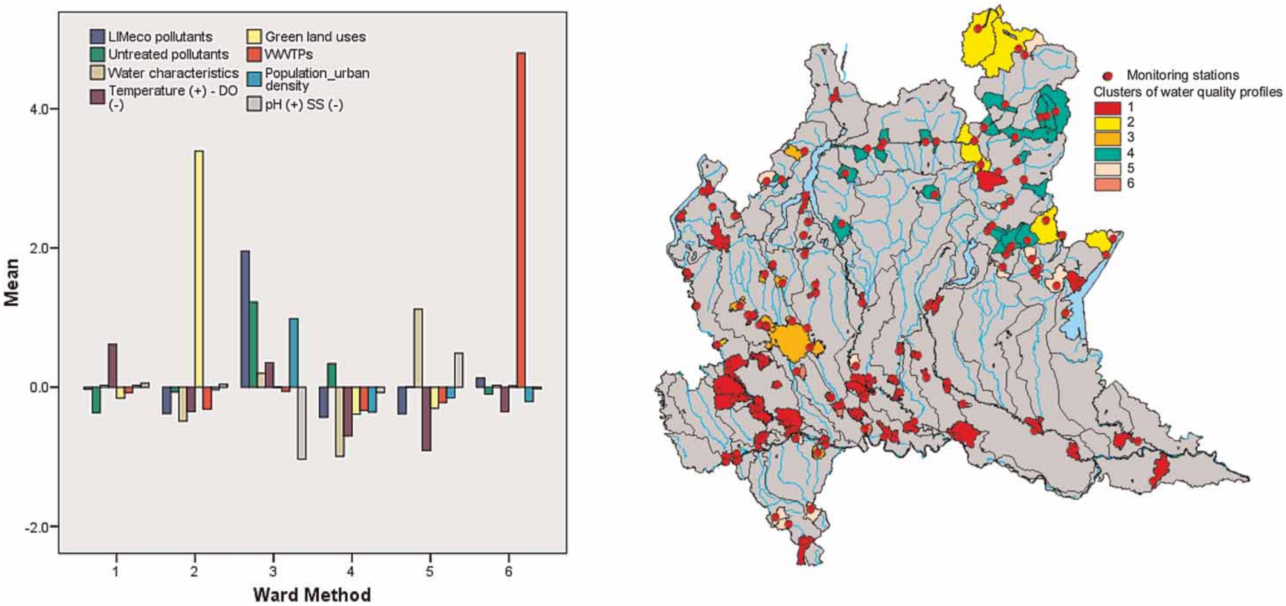

Figure 2 | Cluster characteristics in terms of standardized varifactors (on the left) and shaded (color in online version) on the Lombardy map (on the right). For cluster interpretation refer to text.

cluster 3 (*n*: 11) all the pollutants are higher than the average; this is typical of urban areas with high population densities;

cluster 4 (*n*: 23) all the pollutants are lower than the average with presence of untreated wastewaters higher than the average (typical profile of the prealpine areas);

cluster 5 (*n*: 20) has average water characteristics with the exception of pH and dissolved ions, which are higher than the average;

cluster 6 (*n*: 4) is an outlier group and presents very high concentration of treated wastewater volumes.

Macroinvertebrate assemblages, diatoms and macrophytes are among the mandatory biological monitoring elements indicated in the European WFD.

It is interesting to observe that the three elements of the ecological status correlate perfectly with each other and with LIMeco when considering the whole data set (see Table 5) whereas they do not necessarily correlate when splitting the data set into the six described clusters (see Table 6).

This is because LIMeco is strongly influenced by the major alteration produced by human effluents, which,

Table 5 | Correlation matrix between the three indexes of ecological status and LIMeco

		LIMeco	**Macroinvertebrates**	**Diatoms**	**Macrophytes**
LIMeco	Pearson correlation	1	0.759[a]	0.595[a]	0.753[a]
	Sig. (2-tailed)		0.000	0.000	0.001
	N	120	120	120	16
Macroinvertebrates	Pearson correlation	0.759[a]	1	0.550[a]	0.520[b]
	Sig. (2-tailed)	0.000		0.000	0.039
	N	120	120	120	16
Diatoms	Pearson correlation	0.595[a]	0.550[a]	1	0.315
	Sig. (2-tailed)	0.000	0.000		0.235
	N	120	120	120	16
Macrophytes	Pearson correlation	0.753[a]	0.520[b]	0.315	1
	Sig. (2-tailed)	0.001	0.039	0.235	
	N	16	16	16	16

[a] Correlation is significant at the 0.01 level (2-tailed).

[b] Correlation is significant at the 0.05 level (2-tailed).

All the data are pooled together.

Table 6 | Correlation matrix between the three indexes of ecological status and LIMeco

Cluster		**LIMeco**	**Macroinvertebrates**	**Diatoms**
1	LIMeco	1	0.544[a]	0.472[a]
	Macroinvertebrates	0.544[a]	1	0.367[a]
	Diatoms	0.472[a]	0.367[a]	1
	Macrophytes	0.673[b]	0.449	0.370
2	LIMeco	1	0.666	−0.320
	Macroinvertebrates	0.666	1	−0.628
	Diatoms	−0.320	−0.628	1
	Macrophytes			
3	LIMeco	1	0.898[a]	0.466
	Macroinvertebrates	0.898[a]	1	0.662[b]
	Diatoms	0.466	0.662[b]	1
	Macrophytes			
4	LIMeco	1	0.533[a]	0.231
	Macroinvertebrates	0.533[a]	1	0.489[b]
	Diatoms	0.231	0.489[b]	1
	Macrophytes			
5	LIMeco	1	0.325	0.271
	Macroinvertebrates	0.325	1	0.394
	Diatoms	0.271	0.394	1
	Macrophytes			
6	LIMeco	1	0.607	0.548
	Macroinvertebrates	0.607	1	0.970[b]
	Diatoms	0.548	0.970[b]	1
	Macrophytes			

[a] Correlation is significant at the 0.01 level (2-tailed).
[b] Correlation is significant at the 0.05 level (2-tailed).
The data set is split into clusters.

considering the whole Lombardy territory, shows a strong gradient from the less urbanized areas to the highest urbanization. However, when the relationship between LIMeco and the indexes of ecological status is considered within the clusters, it remains significant only in the clusters of average pollution characteristics. No correlation can be observed in clusters 2, 5, and 6 although their status does not necessarily match the good ecological status required by the WFD.

Finally, quantile regression (Cade & Noon 2003) allows the limiting conditions that may hamper the potential for restoration to be outlined. The percentile selected to define the upper boundary of the data (90th or 95th percentiles are generally chosen to define the upper boundary) may prevent setting unrealistic restoration goals to a natural and pristine biological state in the presence of a gradient of urbanization.

To identify the parameters that more reasonably represent the upper boundary condition for biotic communities, a stepwise multiple linear regression analysis was applied. The stepwise procedure enabled selection of the most significant parameters which correlate with the macroinvertebrate metric index. Table 7 shows the summary statistics of the regression analysis. The stepwise procedure selected as the best set of predictors the parameters N-NH_4, Total-P and COD. Based on these results, quantile regression was applied on all the LIMeco parameters plus COD and BOD. BOD was included by reason of its correlation with COD and DO. Figure 3 shows the standardized values (i.e. Z-scores) of the variables BOD, COD, Total-P, N-NH_4 and 100-DOsat and the corresponding 90th percentile regressions versus the macroinvertebrates index: it can be observed that the

Table 7 | Summary statistics of the stepwise multiple linear regression analysis

Model[a]		Unstandardized coefficients		Standardized coefficients		
		B	Std error	Beta	t	Sig.
1	(Constant)	0.658	0.020		33.186	0.000
	Z-score(N-NH_4)	−0.192	0.026	−0.611	−7.290	0.000
2	(Constant)	0.677	0.020		33.350	0.000
	Z-score(N-NH_4)	−0.574	0.141	−1.826	−4.062	0.000
	Z-score(Total-P)	0.461	0.168	1.235	2.747	0.007
3	(Constant)	0.674	0.020		34.139	0.000
	Z-score(N-NH_4)	−0.504	0.140	−1.603	−3.599	0.001
	Z-score(Total-P)	0.470	0.163	1.259	2.884	0.005
	Z-score(COD)	−0.107	0.043	−0.317	−2.520	0.014

[a] Dependent variable: STAR_ICMi.

The stepwise procedure was terminated at step 3 and identified as best set of predictors N-NH_4, Total-P and COD.

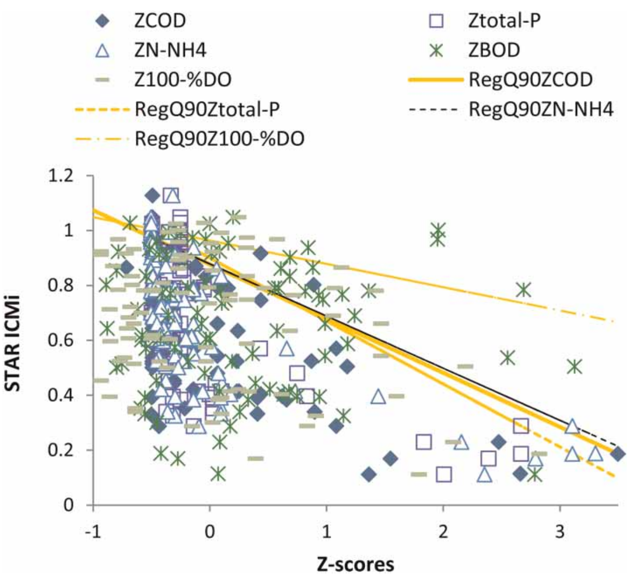

Figure 3 | Quantile regression: the 90th percentile relationship between the Z-scores of the main LIMeco pollutants plus COD and BOD and the STAR_ICMi index.

most limiting pollutants (i.e. the quantile regression with the steeper slopes) are Total-P, N-NH_4, and COD.

Conversely, it should be noted that the 90th quantile regression with % DOsat is the one with the lower slope, outlining how this parameter in most of the cases is not limiting the biotic communities in the study area.

Figure 4 shows the complete range of quantile regression (i.e. 10th, 20th, 40th, 60th, 80th and 90th quantiles) for the most limiting factor according to the 90th quantile regression, i.e. Total-P.

It can be observed that the response of the macroinvertebrate community to Total-P concentration can be highly variable. It should be pointed out that even at the concentration of 0.1 mg/l of Total-P, which according to LIMeco scores is the limit threshold for the good quality status, the boundary conditions at 10th and 90th quantiles correspond respectively to a STAR_ICMi of 0.4 and 0.9, therefore extremely different. Moreover, if this approach makes sense, since it outlines situations where putting the threshold too high (e.g. as far as LIMeco is concerned, the Total-P threshold derives from the 90th percentile of Total-P concentrations in the reference sites) might determine unrealistic restoration goals, its efficacy is still limited by the fact that it does not consider the covariance among the parameters. As Table 7 shows, in fact, when Total-P is considered together with N-NH_4 and COD its correlation with STAR_ICMi turns positive (see B coefficient) and this is the direct consequence of its collinearity with N-NH_4 and COD. This means that when Total-P correlation with the STAR_ICMi index is corrected for the real limiting factors (i.e. N-NH_4 and COD) it turns from negative (i.e. higher concentrations limit the quality status of the biotic community) to positive (i.e. higher concentrations enhance the quality status of the biotic community). This suggests that in most situations the macroinvertebrate community is not affected by Total-P concentrations and its quality might recover if COD and N-NH_4 only are removed, with obvious consequences on the costs for restoration. Following the same rationale, it must be concluded that in cluster 2, 5 and 6 conditions, where the quality status of macropollutants do not correspond to the ecological quality status, focusing the restoration mostly on the removal of these pollutants would just lead to ineffective and costly solutions.

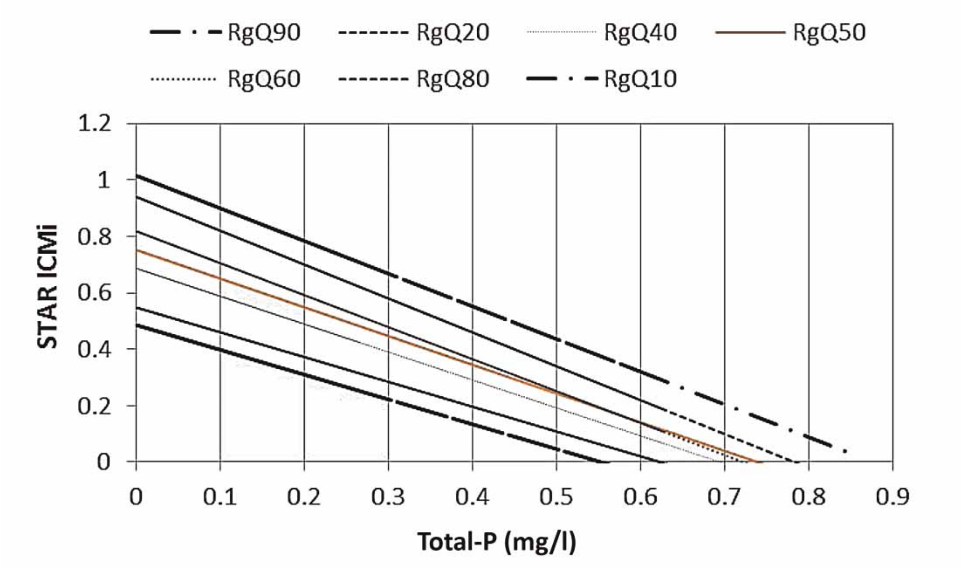

Figure 4 | Quantile regression: the complete set of 10th, 20th, 40th, 60th, 80th and 90th quantiles are shown with respect to Total-P concentration.

CONCLUSIONS

This study shows a process to disentangle the effects of the different stressors. Different profiles of water quality are associated with the dominant stressors and to the corresponding biological status. Disentangling the effects of the different stressors may support more refined management actions and efficient prioritization of scarce resources, providing knowledge-based support for the identification of restoration scenarios that maximize the effectiveness/cost ratio.

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