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Direct measurement of nutrient concentrations in freshwaters with a miniaturized analytical probe: evaluation and validation

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Abstract This work deals with the evaluation of the aqueous concentrations of dissolved reactive phosphorus (DRP), total phosphorus (TP), and ammonium nitrogen (N-NH_4) in surface water by means of direct online instrumentation. A portable, submersible, and automated analyzer designed to measure dissolved and total nutrient concentrations characterized by miniaturization of the entire analytical process was tested against laboratory methods. A total number of 36 water samples of different origin (i.e., rain, river, lake, and sewage waters) were analyzed and used in the comparison of DRP, TP, and N-NH_4 data. Raw data were distributed in a broad range of concentrations: 5–299 μg P/L for DRP, 7–97 μg P/L for TP, and 11–332 μg N/L for N-NH_4. Regression analysis underlined a high significant correlation between the measures of the probe and those of the laboratory ($0.6 < R^2 < 0.9$; $p < 0.001$) and pointed out the effectiveness of the new instrument in representing a broad range of nutrient concentrations.

Keywords Field measurement · Nutrients · Total phosphorus · Water pollution · Miniaturized probe

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Introduction

Continuous measurement of nutrient concentrations is increasingly advocated for both water quality research and management of water bodies and resources (Cassidy and Jordan 2011; Capodaglio et al. 2016a). The dynamics of nutrients in surface waters are characterized, in fact, by high spatial and temporal variability, and their understanding requires adequate planning of monitoring campaigns requiring large numbers of sampling measurements (Horsburgh et al. 2010, Jones et al. 2012). The number of required measures, however, can often exceed the analytical capacity even of the largest and better organized laboratories if carried out with traditional methods.

An approach to overcome this limitation is the use of proxy, or surrogate, variables that are easily measurable in the field, whose values show linear relationships over a broad range of concentrations to a given nutrient (Jones et al. 2011; Salerno et al. 2014). A typical proxy for aggregate dissolved nutrient concentrations is electrical conductivity, while turbidity has shown to be a good tracer of total concentrations, with particular respect to total phosphorus (Stubblefield et al. 2007). Correlation between concentrations of nitrate and spectral extinction parameters yields consistently excellent correlation coefficients, thus nitrates can be easily measured by means of UV-VIS online spectrometers (Capodaglio et al. 2002; Capodaglio 2016). The main limitation of this approach is that the relationship between proxy and nutrient concentrations tends to be site-specific (Grayson et al. 1996; Stubblefield et al. 2007).

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This still implies the need of intensive field campaigns, with consistent analytical efforts, to support a calibration exercise (Viviano et al. 2014), an issue that could be overcome through the use of instrumentation capable of directly measuring nutrient concentrations in the field, analytically. Such equipment, however, was not available until recent years (Horsburgh et al. 2010) and it is still seldom applied. Ideal characteristics of such systems should include high measuring rates (hourly or shorter), robustness, ease of transportation and installation, and availability of remote control facilities (Moscetta et al. 2009).

From a water resources management point of view, estimation of nutrient loads generated in a watershed is certainly one of the most critical issues. This is particularly true when the generated nutrient load sink is a water body characterized by high (hydraulic) retention times, such as lakes or some costal environments, and thus subject to high ecological vulnerability to nutrient loading (Boguniewicz et al. 2006; Carraro et al. 2012). Furthermore, recent studies revealed the existence of very complex relationships between river discharge and nutrient concentrations (Cassidy and Jordan 2011; Bowes et al. 2015); this is especially true in heavily human-impacted environments, where nutrient concentrations are strongly influenced by nutrient load pulses from sewerage networks, particularly due to the impact of combined sewage overflows (Capodaglio et al. 2003; Viviano et al. 2014). The need to approach these studies and their possible solution on a greater level of detail is evident, as it is clear, the implication that a large numbers of measurements, both in frequency and spatial distributions, is needed (Horsburgh et al. 2010).

In this paper, results of an independent study aiming at the verification of the effectiveness and applicability of a commercial-miniaturized chemical analyzer, designed to measure both dissolved and total nutrient concentrations in aquatic environments, are presented. The study can be used as a reference by researchers trying to design similar field studies and was conducted by comparing results from probe monitoring with those obtained from traditional sampling and validated, standard laboratory techniques. The parameters taken into account were as follows: dissolved reactive phosphorus, total phosphorus, and ammonium nitrogen. The study was paired by routine water quality monitoring campaigns by the Water Research Institute-National Research Council of Italy (IRSA) in Brugherio (Milan).

Methods

Sampling

Measurements were taken and samples collected in the basins of three water bodies in Italy that had been the object of IRSA's monitoring for some time (Fig. 1). Sampling sites and watershed codes are listed in Table 1, which also reports the number of collected samples (N) and the mean value of three basic hydrochemical variables: electrical conductivity (Cond), total alkalinity (T_{alk}), and pH. Analyzed samples include rain waters collected both in the open field (rain-open) and below the canopy of trees (i.e., throughfall, rain-THR); river and stream waters (River Masino, Stream Gajum, Stream Segrino, Stream Lambrone, and artificial channel and impoundment waters used for agricultural irrigation) (Channel Molinara); sewage waters (i.e., combined sewage overflow, CSO-Canzo): lake (Lake Pusiano); and reservoir (Lake Occhito).

A total of 36 water samples were collected and analyzed during field monitoring conducted with a direct measurement nutrient probe, described below. In Table 1, samples are listed in ascending order for the electrical conductivity. This variable ranged from 5 (rain-open) to 950 (Stream Cigno) μS/cm 20 °C, underlining a wide range of ionic strengths in the water bodies, with the lowest values related to the samples from River Masino watershed, the intermediated values from the Lake Pusiano watershed, and the highest values from the Lake Occhito watershed. A similar ranking can be observed for T_{alk} and pH, with the lowest buffering capacity and pH values related to the River Masino watershed and the highest related to the Lake Occhito watershed.

While on-site direct measurements were taken, rain and lake samples were collected using standard methodologies as reported in Balestrini et al. (2006) and Legnani et al. (2005), respectively, while simple grab sampling was carried out in the other water bodies. All the samples were stored in polyethylene containers and maintained at 4 °C both during in the transportation and laboratory.

Nutrient measurement probe description

The instrument used is the "Wiz-Probe", an innovative in situ chemical analyzer for nutrient measurements. Its design is based on the patented, well tested, "micro

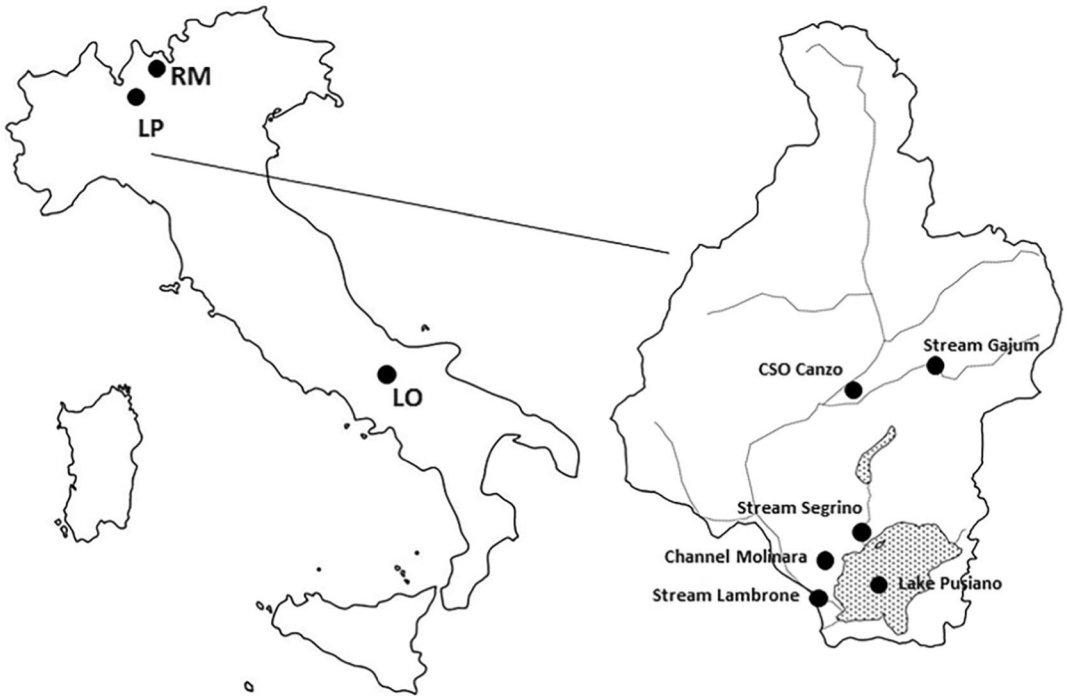

Fig. 1 Location of the three basins sampled in this study (*RM* River Masino basin (46° 14′ 38″ N, 9° 35′ 56″ E); *LP* Lake Pusiano basin (45° 48′ 10″ N; 9° 16′ 16″ E); *LO*=Lake Occhito basin (41° 34′ 52″ N; 14° 56′ 42″ E)) in the Italian Peninsula (*right*) and details of the sampling station location in the Lake Pusiano basin

Loop Flow Analysis" (μLFA) technology developed by SYSTEA SpA (Anagni, Italy) in the mid-1990s (Moscetta et al. 2009; Azzaro 2013, Bodini et al. 2015) that has then been later implemented in miniaturized version in the Wiz-Probe. The latter is characterized by small dimensions (Fig. 2) and includes both a reagent

Table 1 Site and watershed codes, number, and basic hydrochemistry of the analyzed samples

Site	Watershed	*N*	Cond (μS/cm 20 °C)	T_{alk} (meq/L)	pH
Rain-open	RM	3	5	<0.05	5.8
Rain-THR	RM	3	16	<0.05	5.6
River Masino	RM	1	20	0.11	6.7
Lake Pusiano	LP	6	291	3.15	8.0
Stream Gajum	LP	1	306	3.33	8.2
Stream Segrino	LP	2	337	3.18	8.1
Stream Lambrone	LP	3	349	3.50	8.4
Channel Molinara	LP	3	411	3.86	8.2
CSO-Canzo	LP	1	511	5.19	7.6
Tank Finocchito	LO	2	591	4.11	8.5
Lake Occhito	LO	7	624	4.09	8.6
River Fortore	LO	1	731	5.66	8.2
River Tappino	LO	1	743	4.77	8.7
Stream La Catola	LO	1	930	6.42	8.4
Stream Cigno	LO	1	950	5.29	8.5

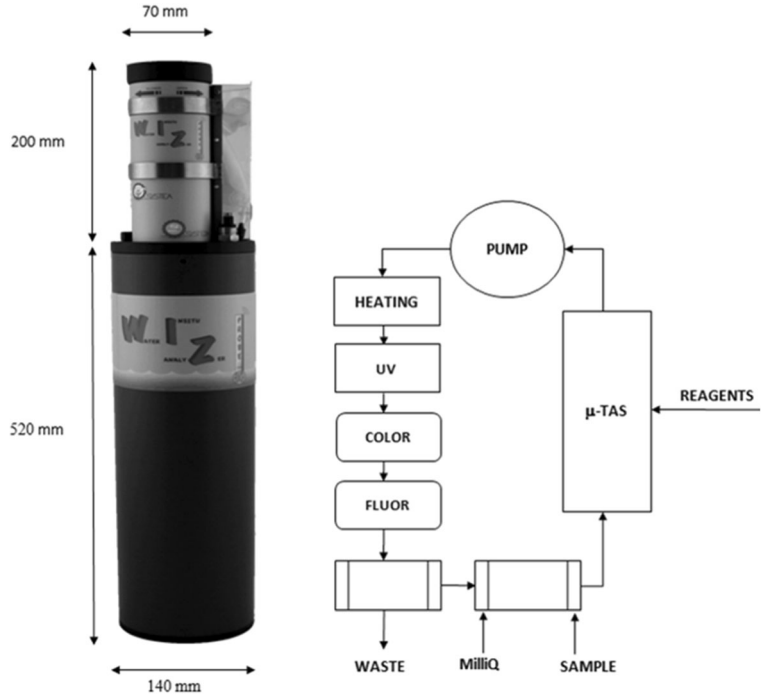

Fig. 2 Wiz-probe (*left*) with scheme of reagent and analytical units (*right*) (SYSTEA 2016)

container and an analytical unit. These communicate with each other through a connecting "umbilical cord" carrying ten liquid lines to deliver reagents and calibration solutions. The cover unit hosts different hydraulic connections for water samples and Milli-Q® water inlet and for the discharge of waste. Waterproof connectors allow the use of an external power supply (12 V, continuous current) and data transfer (RS-232 serial connection). The sealed analytical unit hosts both the electronics and the hydraulic components. The former include auxiliary board, central processing unit, and in/out boards, while the latter consist of peristaltic pump, micro Total Analysis System (μTAS) hydraulic manifold, spectrophotometric flow-cell (length 20 mm), quartz fluorometric flow-cell (length 10 mm), reagent/sample mixer, and heating and UV units. All hydraulic connections consist of 1.6-mm Teflon tubing, Teflon nuts, and ferrules (Moscetta et al. 2009). Figure 3 shows the μLFA hydraulic circuit for direct fluorimetric phosphate analysis.

The probe can be submerged up to a depth of 8 m, runs in multitasking environment, and is programmable with an external personal computer, via a RS-232 serial port. The analytical cycle is activated by the execution of a predefined sequence of macro-commands. The user interacts with the analyzer using a Windows-based software, and a remote control can be achieved through a GSM/GPRS device. Each measured value is stored inside the internal memory along with metadata (e.g., sampling date and time) that allow an accurate description of the measure.

The hydraulic circuit has a volume of 4 mL and is preliminary filled with Milli-Q® water before each measuring cycle. At the beginning of the cycle, the water sample is withdrawn by means of a pump. The required reagents are then sequentially injected (through the μTAS manifold), and mixed with the sample, following conditioning procedures needed for that specific analytical protocol. After completion of the measuring cycle, the hydraulic circuit is washed again with Milli-Q® water.

The probe is designed to measure up to four variables simultaneously, among ammonium, nitrate, total nitrogen (TN), dissolved reactive phosphorus (DRP), and total phosphorus (TP) (Moscetta et al. 2009) and can be programmed accordingly. Ammonia is measured fluorometrically at 370-nm excitation and 420-nm emission by the orthopthaldialdehyde-sulfite reaction (Aminot et al. 2001); this method is highly selective and matrix effects are negligible. DRP (orthophosphate) is commonly measured by using acid molybdate solution and ascorbic acid, and measuring spectrophotometrically the blue color

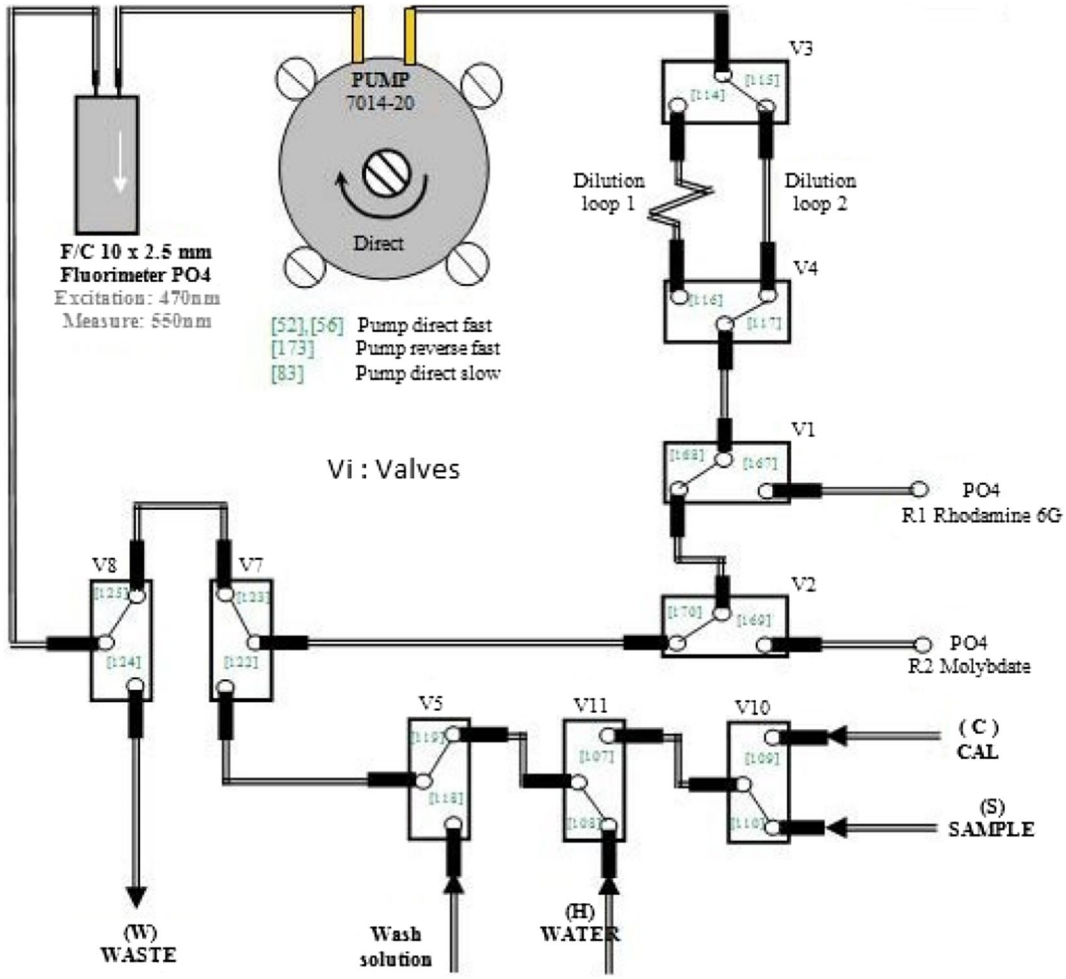

Fig. 3 μLFA hydraulic circuit for direct fluorimetric phosphate analysis (SYSTEA 2016)

of the phosphomolybdenum complex formed at 880 nm (Korostynska et al. 2012), whereas, in μLFA modules, is determined by fluorescence quenching of rhodamine 6G (Taniai et al. 2003).

Nitrate and nitrite are measured using nitrate UV photoreduction and successive determination of reaction products as nitrites. During sample pretreatment, DTPA (diethylenetriamine pentaacetate) and TRIS (tris(hydroxymethyl)aminomethane) buffers are added, and the mixture is then subjected to UV irradiation. Nitrites formed by the photoreduction then react with *N*-(1-naphtyl)ethylendiaminedi-HCl (NED) and sulphanilamide (SAA) in strongly acid medium (HNO_2) to form a pink-colored azo dye measurable at 525 nm (Zhang and Wu 1986). Nitrite is measured spectrophotometrically using NED and SAA as described above (Azzaro 2013).

Laboratory methods

Basic hydrochemical variables (electrical conductivity, total alkalinity, and pH) were analyzed following standard methods (APHA et al. 2000) during the campaign. Total phosphorus (TP) was measured following the method proposed by Valderrama (1981). This procedure involves the addition of oxidizing reagents (potassium peroxodisulphate, boric acid, and sodium hydroxide) to an aliquot of sample, which is then digested in an autoclave per 30 min at 120 °C to oxidize the different forms of phosphorus to orthophosphate. After digestion, a solution of ascorbic acid is added to the aliquot to reduce the free-chlorine produced during the oxidation process. TP is thus measured as dissolved reactive phosphorus (DRP) as reported below. DRP was measured in molecular absorption to an aliquot of the filtered sample, following the methods

proposed by Valderrama (1977). A reducing solution (ascorbic acid, ethylenediaminetetraacetic acid, and formic acid) and other reagents (antimony potassium tartrate, ammonium heptamolybdate, and sulfuric acid) were added to the sample. In the acid environment, the orthophosphate ions react with ammonium heptamolybdate and antimony potassium tartrate to form the ammonium phosphomolybdate complex, in turn reduced to molybdenum blue by the ascorbic acid. The spectrophotometric reading is made at 882-nm wavelength.

Ammonium nitrogen (N-NH_4) was measured by molecular absorption on an aliquot of filtered sample following the method proposed by Fresenius et al. (1988). This procedure exploits the colorimetric reaction between the ammonium and the hypochlorite ions in the presence of the catalyst sodium nitroprusside that determines the formation of the indophenol blue complex measurable at the wavelength of 690 nm after zeroing with Milli-Q® de-ionized water.

The fully automated nutrient measurement cycle lasts 38 min, and the stored reagents capacity allows the probe to carry on about 500 complete cycles once deployed in the field.

Experimental approach and data analysis

All laboratory analyses were conducted at Brugherio IRSA-CNR facilities, using identical analytical equipment, procedures, and the same personnel. Probe measurements were conducted by IRSA-CNR staff in the field. Some of the analyzed samples were excluded from the statistical comparison: specifically, those with laboratory measurements below detection limit (5 μg P/L for DRP and TP and 10 μg N/L for N-NH_4) and those (one for each nutrient species) with outlier concentrations (higher than 400 μg/L). With these criteria, 18, 19, and 23 measurements pairs were subject to statistical comparison for DRP, TP, and N-NH_4, respectively. Differences between probe and laboratory values were evaluated by instrumental deviation (ID) defined as:

$$\mathrm{ID} = (\text{probe value-laboratory value})/\text{laboratory value}^{*}100$$

Value dispersion was evaluated using the coefficient of variation (CV) calculated as the ratio between the standard deviation and the mean values of replicated measures (in percent). Statistical tests and elaborations were carried out using the software R 3.0.2 (http://www.r-project.org/).

Results and discussion

Dataset statistics

Statistics of the study are reported in Table 2, where laboratory (Lab) and probe (Wiz) field-reported values are shown for each investigated species. Taking the laboratory results as reference, the range of concentrations for the three variables is 5–299 μg P/L for DRP, 7–97 μg P/L for TP, and 11–332 μg N/L for N-NH_4. The TP series is thus characterized by a narrower range of concentrations with upper value <100 μg P/L, while maximum values for the other species are close to 300 μg/L.

The similarity of percentile distributions of both probe and laboratory series is indicative of the probe's capability to properly describe the broad range of nutrient concentrations detected in the laboratory, spanning from values typical of uncontaminated waters to values indicative of strong anthropogenic contamination

Probe efficiency

Table 2 shows a marked difference between the median and the mean value in all the data series, with the latter statistics always higher than that of the former. This suggests that data are distributed according to an asymmetric, non-normal distribution, with shift toward the lower values of the series. Application of the Shapiro-Wilk test (Shapiro and Wilk 1965) confirms the non-normality (p value < 0.05) of both laboratory and probe series measurements.

Table 2 Statistics of the measures carried out in this study

	DRP		TP		N-NH_4	
	μg P/L		μg P/L		μg N/L	
	Lab	Wiz	Lab	Wiz	Lab	Wiz
Min	5	8	7	10	11	1
10th percentile	6	9	10	12	13	7
25th percentile	11	12	12	15	28	19
Median	30	36	22	23	40	23
Mean	73	61	28	29	73	55
75th percentile	94	76	37	34	77	69
90th percentile	218	173	48	52	154	143
Max	299	209	97	94	332	283
N	18	18	19	19	23	23

Data were subsequently normalized with natural logarithmic transformation, after which the Shapiro test p value resulted in higher than 0.05, and quantile distribution of the series approximated quite well the theoretical normal quintile distribution (normal Q-Q plot, not shown). This allowed the application of a simple linear model to verify the presence of significant relationships between laboratory and probe measurements. Results of this linear modeling are reported in the three panels of Fig. 4, also reporting the theoretical $x = y$ correlation line indicating perfect agreement between the two series. For all investigated variables, a positive, highly significant correlation ($p < 0.001$) was found, with R^2 between 0.56 and 0.91.

Results presented in Fig. 4 underline a high agreement between laboratory and probe measurements and confirm the capability of the automated analyzer to realistically represent concentrations of the three species over a broad range of values. A closer inspection of the regressions shown in Fig. 4 indicates the probe average tendency to underestimate laboratory measures over the entire range of N-NH_4 concentrations. For both phosphorus species, instead, the tendency to overestimate the lower concentrations and underestimate the higher ones exists. This is well described by the instrumental deviation (ID) trend over the concentration range (Fig. 5).

Differences between probe and laboratory measurements result higher at the lower concentrations (5–10 μg/L), where they reach percentages greater than 100%. As expected, these differences subsequently decrease with increasing nutrient concentrations of all three variables. As already mentioned, instrumental deviation associated to N-NH_4 measurements is always negative. Those associated to both DRP and TP, instead, result positive in the lower concentration range and negative at the higher one. The interpolation curves of DRP and TP intercept the x-axis at concentrations of about 63 and 36 μg P/L, respectively. Equations listed in Fig. 5 allow an estimate of the concentration associated to a given instrument deviation. Instrument deviations of about ± 25% are associated, for instance, to a concentration range of 25–160 μg P/L, and 20–70 for DRP and TP, respectively, while deviations lower than 25% are seen in the range 62–1216 μg N/L, for N-NH_4.

Dispersion of measurements (replicates) was, for both the laboratory and the automated analyzers, higher at low concentrations and lower at high concentrations, following a typical Horwitz curve (Albert and Horwitz 1997). The trend of replicate dispersion (CV) related to the N-NH_4 measurements is reported as example in Fig. 6. Data are represented in logarithmic scale to better identify single data points. Trends of both laboratory and probe measurements appear very similar with an average greater dispersion of the probe replicates.

Results commentary

Eutrophication has become one the primary water quality issues for most freshwater and coastal marine ecosystems around the world, with an always increasing number of documented episodes every year (Kennish and De Jonge 2011). In addition to point (Capodaglio et al. 2015, 2016b) and nonpoint (Lee 1973; Meals 1996; Novotny 2003) nutrient sources control, implementation of suitable analytical tools providing online, unattended, punctual monitoring of river basins appears to be the most feasible management strategy to improve recipient ecosystems quality.

The Alliance for Coastal Technologies (ACT) partnership launched the "Nutrient Sensor Challenge" in 2014,

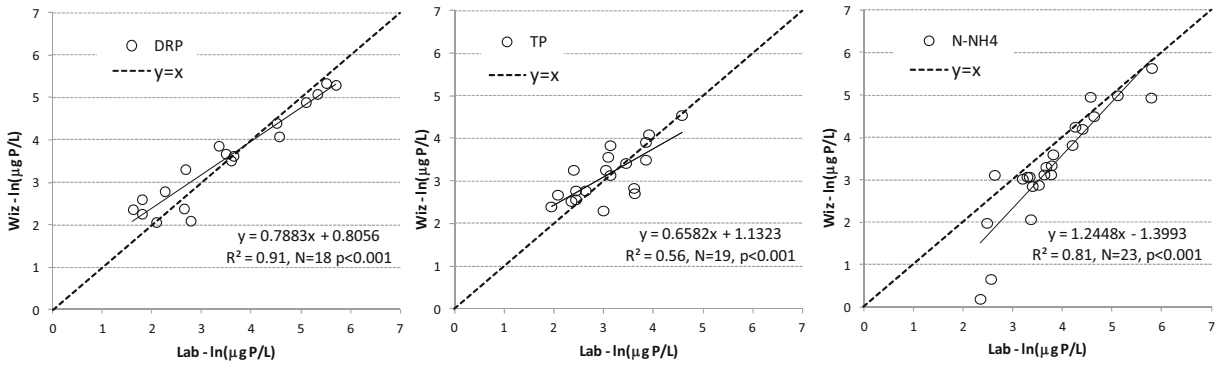

Fig. 4 Relationship between laboratory and probe measurements

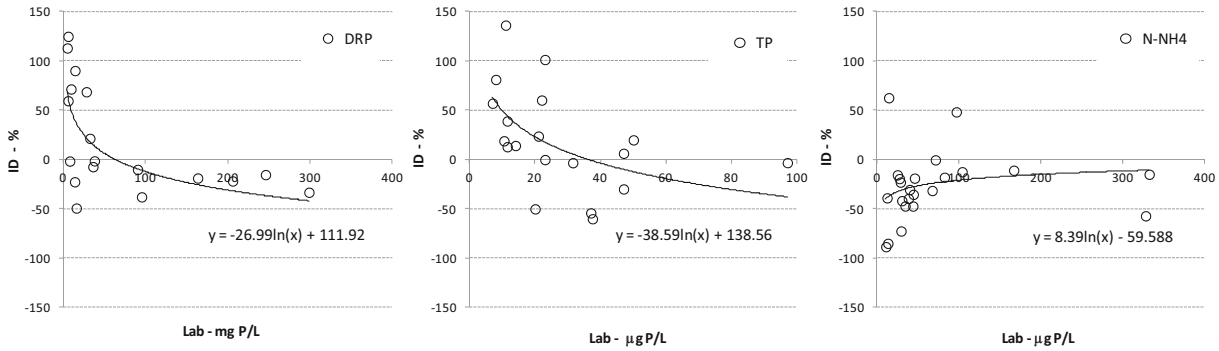

Fig. 5 Trend of instrument deviation (ID) over the nutrient concentration range

through which the needs of actual and potential users of in situ nutrient sensor systems were identified and listed. The most requested sensor technical characteristics concerned analytical performance, including accuracy, precision, ranges, and detection limits (Table 2), followed by deployment life and reliability (ACT 2015). The innovative micro Loop Flow Analysis, implemented in the examined probe, allowing miniaturization of traditional analytical processes can be considered a step toward the fulfillment of those requisites and could help in a deeper understanding of nutrient-related pollution phenomena and the required intervention measures.

The study described, comparing in situ and laboratory measurements validation, shows that the probe can provide fairly accurate and reliable nutrient monitoring in freshwaters. Long-term validation studies should also be carried out.

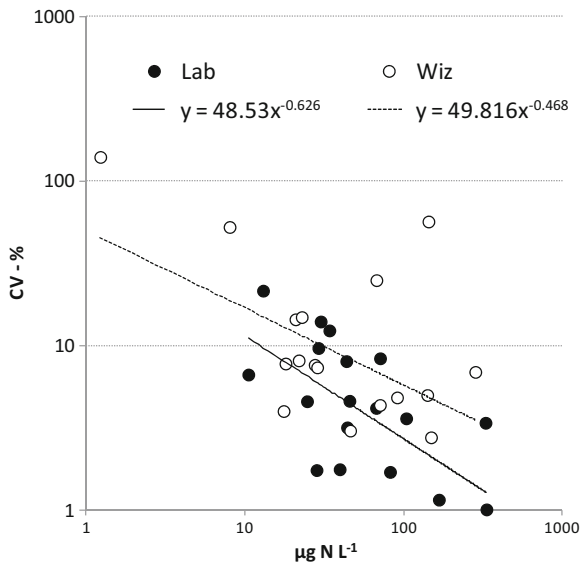

Fig. 6 Dispersion of replicates related to the N-NH_4 measures

Conclusions

Detailed study of the nutrient dynamics in surface waters requires a large numbers of measurements. Situations in which such detailed studies are required are represented by the identification and management of nutrient loads generated in a watershed or by investigations on the development of algal blooms (often triggered by nutrient pulses) in lakes, reservoirs, or costal environments.

Development of equipment capable of carrying out direct, continuous, unattended field measurements of nutrients was until recently facing drastic technical limitations. This is particular true for the measure of total nutrient concentrations, which requires miniaturization of an entire laboratory procedure, including sample digestion, in a portable field instrument.

The results presented in this paper refer to an automated nutrient analyzer of last generation and are certainly encouraging. Measurements carried out with this equipment turned out to be well correlated with measurements performed using traditional, validated laboratory methods in a rather broad range of nutrient concentrations and in different aqueous matrices. Results seem to underline a maximum efficiency of the probe at medium-high concentrations, while at lower concentrations, efficiency is affected by a higher variability that may also affect, however, traditional laboratory measurements. Having thus verified the performance of the analyzer in "static" conditions, future studies should be directed to verify its performance in fully "dynamic", continuously running, long-term field experiments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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