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Editorial Open Access

Microbial Parameters as a Practical Tool for the Functional Characterization and Ecological Status Assessment of Transitional Areas

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Editorial

Transitional areas such as lagoons, ponds, lakes, located at the interface between land and sea, are highly dynamic aquatic ecosystems often characterized by high biological productivity and physical and biogeochemical variability, as well as high naturalistic and economic value [1]. Any environmental policy plan aiming at the preservation, management and development of these coastal ecosystems requires knowledge of their environmental state; therefore, no separate estimations of single environmental parameters, but rather a multi-disciplinary approach can lead to an "holistic" ecosystem understanding.

Lagoons can act either as a sink for organic matter or as sources for adjacent coastal areas, through organic and nutrient export; the accumulation of organic matter is the result of the balance between "*in situ*" production, terrestrial inputs, and processes of decomposition/utilisation and burial in the sediment [2]. Generally, functional characterisation of natural ecosystems relies on knowledge of components, processes and structural relations. The ecosystems biogeochemistry is strongly affected by the taxonomic (specific composition) and metabolic (enzymatic activity profiles) properties of the microbial community; therefore, knowledge of microbial structure and functions is needed for improving our understanding of natural ecosystem dynamics.

In aquatic environments, and especially in lake waters, organic matter is mostly a heterogeneous matrix of polymeric compounds, consisting of proteins (about 50%), polysaccharides (25%) and chemicelluloses (25%) deriving from algae or benthic plants exudates, terrestrial inputs and benthic resuspension. All these compounds are high-molecular weight-polymers (higher than 600 Daltons), that prior to their utilization require preliminary hydrolysis into smaller molecules [3,4]. Therefore microbial utilization of organic polymers depends on the synthesis of exo-(dissolved enzymes) and ecto-enzymes (enzymes attached to microorganisms), usually produced by heterotrophic bacteria, cyanobacteria, eukaryotic algae and heterotrophic nanoflagellates [5]. Thanks to their high abundance, biochemical diversity and quick turnover, microorganisms and in particular heterotrophic bacteria are able to interact quickly with both dissolved and particulate organic matter pools. The decomposition process is generally considered a "bottleneck" for the microbial utilization of both dissolved and particulate organic polymers and the regeneration of inorganic nutrients in aquatic environments [6,7]. Microbial decomposition and heterotrophic utilization of organic matter have significant implications on the chemistry and energy fluxes as well as on the whole ecosystem functioning [8-11].

In coastal ecosystems, heterotrophic bacteria-organic matter interactions are further complicated by abiotic and biotic forcing [12]. In fact, the synthesis and activity of enzymes depend on the physiological state of the microbes and on the trophic conditions of the environment [13]. Micro-organisms respond rapidly to changes in nutrient and substrate availability - which frequently occur in coastal ecosystems - modulating their enzymatic profiles in relation to new organic polymers and, in turn, changes in the specific composition of the microbial community affect the metabolic profiles of microbes and consequently their contribution to decomposition process [14].

Simultaneous studies of microbial activities and particulate organic matter give useful insights about changes in microbial metabolism in response to trophic variability of water bodies. Functional information on the ecological status could contribute to plan appropriate environmental management strategies in the framework of a sustainable use of natural resources [15-18]. Particularly, three microbial ectoenzymes (leucine aminopeptidase, LAP; beta-glucosidase, b-glu; alkaline phosphatase, AP) are mostly involved in organic matter decomposition. LAP is an enzyme associated with the decomposition of protein-derived polymers, although not directly involved in the organic matter remineralization [7]. It is a very widespread enzyme, synthesized by bacteria and cyanobacteria, phytoplankton and zooplankton; LAP is involved in the decay of particulate matter composed of living organisms or nonliving materials, such as faecal pellets etc. [19]. B-glu is an enzyme specific for the hydrolysis of cellobiose, a component of polymers such as cellulose and mucopolysaccharides; therefore it is involved in the decomposition of refractory vegetal debris, derived from "*in situ*" autochthonous production or from allochthonous production. Other than bacteria, also zooplankton and eukaryotes (fungi, diatoms, etc.) have shown to be important producers of extracellular glycosidic activities [20-22]. AP is an enzyme involved in the mineralization of organic phosphates with regeneration of phosphate; both bacteria and phytoplankton contribute to its synthesis [23]. In eutrophic environments, AP is produced in great amounts when inorganic P becomes limited, suggesting its regulation by derepression mechanisms [24] and supporting its use as an indicator of P deficiency of phytoplankton and bacterial assemblages [23,25].

As most of the extracellular enzymes are adaptative and their synthesis and activity is strongly affected by environmental and biological factors [5,13], the relative importance of LAP, b-glu and AP reflects differences in the relative composition of organic matter, in terms of amounts of proteins, polysaccharides and organic phosphates, respectively. The monitoring of prokaryotic properties improves the classification of the ecological status and provides a tool comparable to current indicators of environmental quality, but at a lower cost. Some examples are reported here.

The trophic state of an aquatic ecosystem has been traditionally characterized through the determination of nutrients, together with chlorophyll-a concentration and water transparency, without considering the important role played by the inhabiting microbial community. Through enzyme measurements, Caruso et al. [26] characterized the ecological status of some Mediterranean ponds. A marked biogeochemical diversification of these environments was found in relation to their organic and nutrient loading; higher values and greater variability of microbial activity were found in the most eutrophic lakes (LAP: 192.78 ± 102.11 and 294.34 ± 245.88 nmol Leucine l-1 h-1;b-glu: 45.1 ± 117.42 and 37.8 ± 65.5 nmol Glu l-1 h-1; AP: 209.0 ± 262.21 and 280.77 ± 452.0 nmol PO4l-1 h-1, respectively) compared to the oligotrophic ones (LAP: 118.36 ± 34.72 nmol Leucine l-1 h-1; b-glu: 5.71 ± 8.50 nmol Glu l-1 h-1; AP: 62.35 ± 108.54 nmol PO4l-1h-1, respectively). Moreover, microbial activity rates were higher in summer, suggesting that temperature and trophic supplies stimulated the biological decomposition of organic polymers and active nutrient recycling. Enzyme activities, and particularly LAP, correlated significantly with the trophic parameters (particulate organic matter or chlorophyll-a).

There are many studies where enzyme activities are related with the trophic conditions of water bodies [6,27-29]. This relationship is explained by the fact that high-molecular-weight substrates affect microbial ectoenzyme activity, therefore the activity of some enzymes may correlate with the trophic gradient. According to this consideration, Chrost and Siuda [18] suggested the determination of microbial activity as an indirect proxy for assessing the trophic state in lakes, proving the existence of a tight coupling between the trophic conditions and the extent of microbial processing over organic matter (production, transformation and degradation) in the pelagial zone of lakes located along an increasing gradient of eutrophication.

In three coastal lagoons Manini et al. [2] showed that organic matter composition plays a key role in the ability of microbial loop to channel C-biomass to higher trophic levels; indeed, quantity and quality of sediment organic matter controlled the rates of organic matter degradation, turnover rates (through breakdown of large macromolecules) and utilisation by benthic heterotrophic organisms (through bacterial C production). Also Pusceddu et al. [30] - in the framework of the NITIDA project (New Indicators of Trophic state and environmental quality of marine coastal ecosystems and transitional environments) - demonstrated the close links among trophic state, ecosystem efficiency, and biodiversity in transitional ecosystems and concluded that the assessment of the environmental quality of transitional ecosystems should be based upon a battery of trophic state indicators of ecosystem functioning, efficiency, and quality, among which the prokaryote efficiency in exploiting enzymatically degraded organic Carbon has a preminent role.

Mazzola et al. [31] included the extracellular enzymatic activity rates as functional indicators in a panel of bioindicators (trophic and prokaryotic parameters, benthic invertebrates) to discriminate the environmental quality of some Sicilian transitional ecosystems. Moreover, Caruso et al. [32] explored the possibility to apply an integrated (trophic+ microbial) approach to contribute to the implementation of the Water Framework Directive (WFD), in the perspective of establishing quality criteria which could be used for the transitional waters classification and transferred to similar water bodies. In this study, trophic (POC, Chl-a) and microbial (LAP, AP) parameters described well the different metabolic functionality of the studied water bodies; therefore, microbial parameters, such as microbial activity measurements - in addition to those already included in the WFD - were proposed as a promising approach to better characterize the ecological status of the transitional systems. On the other hand, this new integrated approach provided information comparable to that given by indices conventionally used for the assessment of trophic state (i.e. TRIX); nevertheless, microbial variables described a more comprehensive scenario of the trophic dynamics of transitional water systems and allowed a more efficient characterization.

More recently, Caruso et al. [33] have reviewed the use of enzyme activities as proxies of trophic parameters. Significant seasonal and interannual variations in the patterns of microbial activity, in relation to temperature, dissolved oxygen and trophic changes were found in a brackish ecosystem [34]. Results obtained from several surveys have indicated significant correlations between enzyme activity rates and particulate organic matter or chlorophyll-a used as descriptors of the trophic state and productivity of aquatic ecosystems; consequently, the assay of LAP and AP has been suggested as a suitable and quick approach to discriminate different water bodies according to their trophic state. Furthermore, Sims et al. [35] have recently proposed a multi-metric index which takes into account microbial indicators to assess wetland health condition and to set up appropriate management and restoration strategies.

During a two-years cycle of observations in a brackish ecosystem (Cape Peloro, Sicily), Zaccone et al. [36] studied whether microbial parameters could provide functional ecological information to monitor the environmental health. Microbial indicators (vibrios abundance and microbial activities) were found to be highly responsive to the spatial and seasonal changes of environmental parameters, such as temperature and trophic conditions. Positive correlations between temperature and heterotrophic production, LAP, AP, particulate organic carbon and nitrogen were observed in Ganzirri Lake, indicating a direct stimulation of the warm season on the heterotrophic prokaryotic metabolism. The global warming might stimulate the growth of opportunistic Vibrio spp., resulting in an improved degradation of labile organic matter. The relationships found at seasonal scales between these bacteria and trophic conditions suggested the combined use of vibrios abundance and microbial activities as indicators to monitor the organic matter turnover within lagoon areas.

Incorporation of microbial variables into the conventional classification schemes developed for assessing the ecological status - like Indicators and Methods for the Ecological status assessment under the WFD, EUR 22314EN - could also provide information on ecosystem functioning. Although the validation of the proposed integrated approach on a broader scale is necessary, it may provide users with a simple tool that gives a comprehensive view of microbial processes and their effects on water biogeochemistry and productive processes. In conclusion, the microbial parameters (both abundance and activities) can be considered as a simple promising tool for assessing the trophic status and environmental quality of transitional areas, which could also be applied to other water bodies.

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