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Stable isotope-based statistical tools as ecological indicator of pollution sources in Mediterranean transitional water ecosystems

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ABSTRACT

N-stable isotope analysis of macroalgae has become a popular method for the monitoring of nitrogen pollution in aquatic ecosystems. Basing on changes in their $\delta^{15}N$, macroalgae have been successfully used as biological traps to intercept nitrogen inputs. As different nitrogen sources differ in their isotopic signature, this technique provides useful information on the origin of pollutants and their extension in the water body. However, isotopic fractionation potentially resulting from microbial nitrogen processing, and indirect isotopic variations due to effects of physicochemical conditions on algal nutrient uptake and metabolism, may affect anthropogenic N isotopic values during transportation and assimilation. This in turn can affect the observed isotopic signature in the algal tissue, inducing isotopic variations not related to the origin of assimilated nitrogen, representing a "background noise" in isotope-based water pollution studies.

In this study, we focused on three neighbouring coastal lakes (Caprolace, Fogliano and Sabaudia lakes) located south of Rome (Italy). Lakes were characterized by differences in terms of anthropogenic pressure (i.e. urbanization, cultivated crops, livestock grazing) and potential "background noise" levels (i.e. nutrient concentration, pH, microbial concentration). Our aim was to assess nitrogen isotopic variations in fragments of *Ulva lactuca* specimens after 48 h of submersion to identify and locate the origins of nitrogen pollutants affecting each lake. $\delta^{15}N$ were obtained for replicated specimens of *U. lactuca* spatially distributed to cover the entire surface of each lake, previously collected from a benchmark, unpolluted site. In order to reduce the environmental background noise on isotopic observations, a Bayesian hierarchical model relating isotopic variation to environmental covariates and random spatial effects was used to describe and understand the distribution of isotopic signals in each lake.

Our procedure (i) allowed to remove background noise and confounding effects from the observed isotopic signals; (ii) allowed to detect "hidden" pollution sources that would not be detected when not accounting for the confounding effect of environmental background noise; (iii) produced maps of the three lakes providing a clear representation of the isotopic signal variation even where background noise was high. Maps were useful to locate nitrogen pollution sources, identify the origin of the dissolved nitrogen and quantify the extent of pollutants, showing localized organic pollution impacting Sabaudia and Fogliano, but not Caprolace. This method provided a clear characterization of both intra- and inter-lake anthropogenic pressure gradients, representing a powerful approach to the ecological indication and nitrogen pollution management in complex systems, as transitional waterbodies are.

1. Introduction

Anthropogenic nitrogen loading of aquatic coastal environments significantly impacts ecosystem structure and functioning, leading to increased productivity, eutrophication, changes in microbial abundance and changes in both producer and consumer community composition (Duarte, 1995; Morand and Merceron, 2005; Rafaelli et al., 1998; Vailela, 1992; Valiela et al., 1997).

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As coastal ecosystems, Mediterranean coastal lagoons are semi-closed and relatively small transitional water bodies, representing important but fragile habitats (Basset et al., 2006; Levin, 2001), which can be particularly affected by surrounding human activities. Direct and indirect anthropogenic nutrient loadings can arise from many activities, including treated and untreated sewage discharge, aquaculture, agriculture and livestock grazing, making it difficult to unambiguously determine the nutrient sources and their related impact on ecosystems (Grant et al., 1995; Jones et al., 2001; Orlandi et al., 2014). N-stable isotopes (^{15}N:^{14}N, expressed as $\delta^{15}N$) are increasingly used to investigate nutrient loading pathways in aquatic ecosystems (Costanzo et al., 2001; Dailer et al., 2010; di Lascio et al., 2013; Jones et al., 2001; Risk et al., 2009; Rožič et al., 2014). Atmospheric (natural) nitrogen can be distinguished from fertilizer and sewage-derived nitrogen on the basis of $\delta^{15}N$ values (Dailer et al., 2010; Macko and Ostrom, 1994; Owens, 1987; Risk et al., 2009), with sewage-derived/organic N being characterized by higher $\delta^{15}N$ values (around +6 to +38‰) than inorganic/fertilizer-derived nitrogen (around −4 to +4‰) (Dailer et al., 2010; Macko and Ostrom, 1994). The presence of different N inputs can thus be detected by changes in the isotopic signature of algal tissues in organisms transferred from an undisturbed location to the water body under study and allowed to uptake the dissolved allochthonous nitrogen (Costanzo et al., 2001, 2005; Jones et al., 2001; Orlandi et al., 2014).

Assuming that macroalgae reach isotopic equilibrium with the assimilated nitrogen source(s), human-derived nitrogen can be directly detected based on changes in the $\delta^{15}N$ of macroalgae, and the allochthonous N input can be mapped by means of spatial analysis based on observed isotopic values (Costanzo et al., 2001). The isotopic values of nitrogen sources can be taken from the literature or measured at the origin of each N source potentially affecting the water body (Dailer et al., 2010). However, isotopic fractionation (i.e. discrimination between ^{15}N and ^{14}N during biochemical reactions) potentially resulting from ammonia volatilization (Heaton, 1986), nitrogen processing by bacteria (Leheman et al., 2003; Macko and Estep, 1984) and microalgal nutrient recycling in the water body (Pennock et al., 1996; Waser et al., 1998) may affect anthropogenic N isotopic values during transportation and processing (Dailer et al., 2010). Moreover, indirect isotopic effects can arise depending on nutrient concentrations (Pennock et al., 1996; Teichberg et al., 2008), nitrogen forms (Mariotti et al., 1981; Waser et al., 1998), temperature, pH and oxygen concentration, since these can all affect microbial activity and algal metabolism and nutrient uptake (Azov, 1982; Jones and Hood, 1980; Leheman et al., 2002; Rhee, 1978; Teichberg et al., 2007), representing background noise in isotope-based water pollution studies, i.e. inducing isotopic variations in algal tissue which are not related to the origin of assimilated nitrogen, potentially leading to erroneous interpretation of isotopic results. Such environmental background noise could be particularly influent in highly dynamic systems, as transitional aquatic environments and coastal lakes are. The relatively high degree of openness (Basset et al., 2006) of these environments makes them particularly sensitive to multiple and interacting stressors impacting the catchment area, with such elevated natural variability in both the environmental conditions and disturbance regimes potentially obscuring human-derived disturbance when occurring at low levels (Cloern, 2001; Vermeulen et al., 2011).

In this context, the spatial arrangement of point and non-point anthropogenic nutrient inputs, as well as their extent in the water body, may be particularly difficult to determine, complicating the discrimination between anthropogenic and natural variations in the measured physicochemical and biological parameters. In this case, modelling may be necessary in order to remove such noise from the observed isotopic values, allowing a reliable use of isotopic data to determine the origin of the dissolved nitrogen, the location of the allochthonous nitrogen inputs and their extent in the water body, with important implications for ecosystem management and conservation. Bayesian statistics is becoming increasingly popular in the ecological community. Using the words of Clark (2005) “advances in computational statistics provide a general framework for the high-dimensional models typically needed for ecological inference and prediction. Hierarchical Bayes represents a modelling structure with capacity to exploit diverse sources of information, to accommodate influences that are unknown (or unknowable) and to draw inference on large numbers of latent variables and parameters that describe complex relationships”. Then, the Bayesian paradigm allows us to merge theory with mechanistic understanding and empirical evidence, to assimilate diverse sources of information and to accommodate complexity often characterizing ecological datasets (Clark, 2006; Clark and Gelfand, 2006). It allows us to estimate the many contributions to the global uncertainty given by several sources, to model prior knowledge and to extract data information in a reliable way, representing a potentially useful approach with which complement isotopic field measurements by accounting for isotopic variations driven by environmental “noise” in pollution-monitoring studies. The Bayesian approach is not new in the statistical modelling of stable isotopes, having been successfully adopted in many protocols to determine animal diet using univariate and multivariate mixing models (Calizza et al., 2012; Erhardt and Bedrick, 2013; Hondula and Pace, 2014; Jackson et al., 2011; Phillips and Gregg, 2001; Richoux et al., 2014). Recently, the increasing interest in Bayesian statistics has led to the development of several tools for specific ecological analyses in the R environment (Jackson et al., 2011; Parnell et al., 2010) and to interesting debates, in particular, in the solving of under-determined stable isotope mixing systems, discussing whether adopt probabilistic approaches in a Bayesian framework (Semmens et al., 2013) or graphical approaches not allowing for probabilistic statements (Fry, 2013).

In the present study, we analysed isotopic changes occurring in a green macroalga, *Ulva lactuca*, deployed in three adjacent coastal lakes in central Italy that are known to differ both in anthropogenic pressure and in potential background noise, i.e. both abiotic and biotic factors potentially affecting isotopic results regardless of differences in anthropogenic nitrogen inputs. Based on environmental and isotopic data, we built a linear mixed-effects model (Zuur et al., 2009) to account for the uncertainty potentially affecting isotopic changes including background noise, potential measurement errors, replicate variability and spatial autocorrelation. The model was estimated in the Bayesian framework and model residuals (i.e. differences between observed and estimated isotopic changes at each sampling point within a given lake) were mapped and used for subsequent analyses. The novelty of this approach lies in its definition of a statistical model for stable isotopes that removes confounding factors from the recorded measures and allows for robust evaluation of the associated uncertainty. According to this, our aim was (i) to build an analysis protocol for isotopic data able to remove the effects of possible confounding factors and (ii) to apply this analysis protocol to the isotopic data in order to obtain reliable intra- and inter-lake comparisons and a robust description of the occurrence, spatial arrangement and extent of the anthropogenic nitrogen inputs affecting each lake.

2. Material and methods

2.1. Study areas

The three study areas were the lakes of Caprolace, Sabaudia and Fogliano (Latina, Italy), located on the Tyrrhenian coast of the Lazio region (42°28′00″N, 12°51′00″E) (Fig. 1). The maximum depths are