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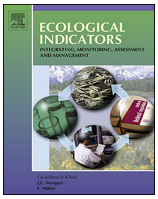

Original Articles

Stable isotopes in helophytes reflect anthropogenic nitrogen pollution in entry streams at the Doñana World Heritage Site

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ABSTRACT

Nitrogen (N) loading from anthropogenic activities is contributing to the eutrophication and degradation of wetlands worldwide. Doñana (southwestern Spain), includes a dynamic marshland protected as a UNESCO World Heritage Site, which has a catchment area exposed to increasing N inputs from intensive agriculture and poorly treated urban wastewaters. Identifying the sources of N entering this iconic wetland complex is vital for its conservation. To this end, we combined multiyear (2014–2016), spatially-explicit data on N concentration in water samples with measurements on the relative abundance of N stable isotopes ($\delta^{15}N$) in *Bolboschoenus maritimus* and *Typha domingensis,* two dominant helophytes (i.e. emergent macrophytes) in the Doñana marsh and entry streams. Overall, plant tissues from entry streams showed higher $\delta^{15}N$ values than those from the marsh, particularly in those streams most affected by urban wastewaters. Isotopic values did not differ between plant species. Water samples affected by isotopically-enriched urban wastewaters and other diffuse organic N inputs (e.g. livestock farming) had relatively high Dissolved Inorganic Nitrogen (DIN) concentrations. In contrast, in streams mainly affected by diffuse N pollution from greenhouse crops, high DIN values were related to isotopically-depleted N sources (e.g., inorganic fertilizers). Thus, helophytes, in combination with other parameters such as N concentration in water or land cover, can be valuable indicators of anthropogenic pressures in Mediterranean wetlands. Helophytes have widespread distributions, and can be readily sampled even when water is no longer present. However, identification of specific N sources through helophyte $\delta^{15}N$ values is limited when key potential N sources are isotopically undistinguishable (e.g. fertilizers vs. atmospheric sources).

1. Introduction

Biogeochemical cycles have been severely altered worldwide by the over-enrichment of aquatic systems with nutrients, especially nitrogen (N). Human pressures, such as increasing use of chemical fertilizers in agriculture or land urbanization, are major and increasing causes of these alterations (Galloway et al., 2008; Tilman et al., 2002; Vitousek et al., 1997).

Wetlands play a key role in regulating the N cycle through different processes such as N sequestration (e.g. biomass production or sediment burial) or N removal (e.g. as N_2 by denitrification) (Costanza and D'Arge, 1997; Jordan et al., 2011; Kingsford et al., 2016). These processes represent a valuable ecosystem service both for society and wetlands, reducing the impact of excessive N inputs which otherwise would cause eutrophication, with adverse effects including cyanobacterial blooms, hypoxia, expansion of floating plants and, ultimately, loss of biodiversity (Compton et al., 2011; Green et al., 2017; Jenny et al., 2016; O'Neil et al., 2012). However, loss and degradation of natural wetlands is ongoing (Davidson, 2014), with major consequences for N regulation and other ecosystem services (Millenium Ecosystem Assessment, 2005).

N excess can originate from a variety of anthropogenic and natural processes. Point sources of excessive N loadings (e.g. chicken farms or wastewater treatment plants (WWTP)) are relatively easy to identify and manage (e.g. Carey and Migliaccio, 2009). In contrast, diffuse N-sources (e.g. arable agriculture, atmospheric deposition) are more difficult to identify and control due to their uneven and widespread distribution within watersheds (Carpenter et al., 1998). Knowledge on the origin and spatial distribution of different N-sources is vital for effective management of N surplus in aquatic ecosystems.

Abbreviations: DIN, Dissolved Inorganic Nitrogen; TN, Total Nitrogen; WHS, World Heritage Site; WWTP, Waste Water Treatment Plant

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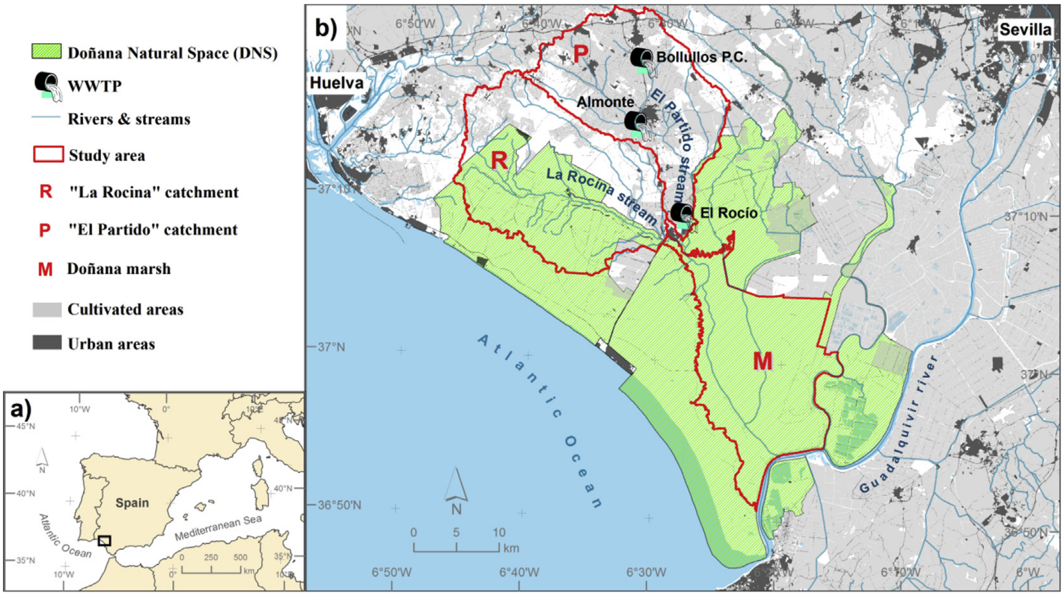

Fig. 1. Location of (a) the Doñana wetlands in western Europe and (b) the limits of the Doñana Natural Space (DNS), the marsh (M) and the two catchment areas for the streams included in this study ("La Rocina" (R) and "El Partido" (P)). Two major anthropogenic pressures in this area are represented in the map (agriculture as cultivated areá and urban pollution as WWTṔ (Waste Water Treatment Plants). Boundaries of "La Rocina" and "El Partido" catchments were delineated using a five metres digital terrain model (MDT05-PNOA) through digital aerial photogrammetry and automatic stereoscopic correlation by the Spanish National Geographic Institute (http://pnoa.ign.es/). Marsh boundaries were delinated using Landsat time series inundation masks and photo interpretation. This work was carried out by the Remote Sensing Lab (LAST) at Doñana Biological Station (EBD-CSIC, Seville).

Ratios of stable N isotopes (^{15}N/^{14}N, commonly expressed as δ^{15}N in ‰) vary among different N sources, providing a useful tool to identify the origin of N in aquatic systems (Heaton, 1986; Michener and Lajtha, 2007). For example, human wastewaters and animal waste N are typically enriched in δ^{15}N (10–20‰), while synthetic inorganic fertilizers have lower δ^{15}N values (-3 to 3‰) because they are derived from atmospheric nitrogen fixation (δ^{15}N $-$values close to zero). Besides the specific N isotopic composition of different sources, common biogeochemical processes in aquatic systems (e.g. nitrification, denitrification, assimilation, fixation and mineralization) may also influence the δ^{15}N values of N compounds. For example, nitrate removal by denitrification results in isotopic enrichment of the heavier isotope (^{15}N) due to isotopic fractionation, thus increasing δ^{15}N values of residual nitrate (Mariotti et al., 1981; Minet et al., 2017). Therefore, the relative abundance of N isotopes (δ^{15}N) in N compounds is the result of mixed N sources and fractionation processes.

Numerous studies have monitored anthropogenic N loading from watersheds into coastal or inland waters by measuring δ^{15}N values in different biotic (e.g. plants, animal tissues) and abiotic (e.g. inorganic N, water) indicators (Cole et al., 2004; Karube et al., 2010; Kaushal et al., 2011; Vander Zanden et al., 2005). Aquatic plants are attractive indicators for tracing N inputs as they assimilate and/or fix N from the surrounding environment, integrating isotopic variability both spatially and temporally and thus reducing noise (Bannon and Roman, 2008; Cole et al., 2004; Kohzu et al., 2008; McIver et al., 2015; Wang et al., 2015). This may be particularly useful in Mediterranean wetlands, which are subject to high temporal variability in flooding patterns (Green et al., 2017), and subsequently in the sources and concentrations of N at a given moment of time. For instance, heavy rainfall events typically cause pulses of nutrients and organic matter in streams from catchment runoff (Bernal et al., 2013), or storm-water overflows from urban areas (Masi et al., 2017).

Aquatic plants can show a wide range of δ^{15}N values (15 to $+20$‰) depending on the available N sources, environmental conditions and physiological features (Kendall et al., 2008). For example, δ^{15}N values in submerged plants are useful indicators of wastewater inputs in temperate estuaries (Cole et al., 2004; McClelland et al., 1997; Savage and Elmgren, 2004).

Doñana, in south western Spain, is one of the most important wetland complexes in Europe and in the Mediterranean region, and is partly protected as a UNESCO World Heritage Site (WHS) (Green et al., 2018). However, these wetlands are under threat due to local human pressures and regional climate perturbations that act together, compromising water quantity and quality (Green et al., 2017). Impacts mainly originate outside the boundaries of the WHS, where economic development has been particularly intense in recent decades (Green et al., 2016; Serrano et al., 2006). Despite their importance, there is a lack of basic knowledge on the sources and levels of nutrient inputs entering the Doñana marshes (Espinar et al., 2015).

The goal of this study was to explore the variability of δ^{15}N values measured in helophytes (emergent aquatic plants) and N concentrations in surface waters to identify the major land-derived N sources and spatial distribution of N loading in the Doñana wetland complex. We compared δ^{15}N values measured in the two helophyte species (*B. maritimus* and *T. domingensis*) and N concentrations in entry streams and in the WHS marsh. We did this during two hydroperiods with contrasting precipitation patterns. We assessed whether the isotopic variation in plants and the N concentration in surface waters were higher in streams, owing to a higher impact of anthropogenic activities in the watersheds. We expected these parameters to be lower in the protected marsh due to the greater distance from intensive anthropogenic activities in the watersheds, and the strong N mitigation capacity of helophytes and microbial processes in the marsh (e.g. denitrification) (Hinshaw et al., 2017; Tortosa et al., 2011).

We also considered whether the stream in the watershed with the highest level of agricultural activity and urbanization ("El Partido") had higher δ^{15}N values and DIN concentrations. We expected this owing to the influence of urban wastewaters, and also due to the highly degraded state of the riparian vegetation, which is likely to reduce the N buffering capacity of the stream in response to diffuse N inputs from agricultural and livestock farming practices (Borja et al., 2009; Pinay et al., 2018).