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Effects of overabundant nitrate and warmer temperatures on charophytes: The roles of plasticity and local adaptation

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ABSTRACT

Global change effects, such as warming and increases in nitrogen loading, alter vulnerable Mediterranean aquatic systems, and charophytes can be one of the most affected groups. We addressed the possible interaction between these factors on two populations of the cosmopolitan charophytes *Chara hispida* and *Chara vulgaris*. Populations were taken from two different environments, a nitrate-poor mountain lake and a nitrate-rich Mediterranean coastal spring. The laboratory experiment had a 2 × 2 factorial design based on two nitrate levels (similar to and double the local conditions) and two temperatures. Increased temperatures favoured the growth of the four populations, but an increase in nitrate did not have any effect on their growth or architecture. Both species took up and stored more nitrogen (measured as %N in plant tissue) when more nitrate was supplied, and warming favoured this increase in %N and, consequently, in N:P ratio. The effects of both factors depended on the local conditions where the populations originated and on the species. *Chara vulgaris*, a pioneer species, exhibited more phenotypic plasticity than *C. hispida*, and its ecotype from the coastal spring was better adapted to changes in temperature and nitrate level. These differential responses to warming conditions and nitrate pollution may modify charophyte diversity, which might be reflected in ecosystem performance, a matter of concern in vulnerable Mediterranean water bodies where these species co-occur.

1. Introduction

Global warming caused by current climate change and the increase in nitrogen input, with impacts on the biosphere, are currently well-documented processes (Lake et al., 2000). Their combination is especially noteworthy in the Mediterranean region (Moss et al., 2011), where the increase in temperature will promote higher evaporation rates, which, combined with a decrease in precipitation, will reduce the depth of the water column in freshwater bodies (IPCC, 2014). Such a decrease in water resources will be especially severe in this region, where intensive agriculture and the overabundant use of fertilisers, such as nitrate, have traditionally existed. The interactive effects of climate change and eutrophication in Mediterranean areas have been a matter of concern for a decade (Giorgi and Lionello, 2008; Jeppesen et al., 2011). Dramatic predictions have been made for Mediterranean countries, where freshwater ecosystems are often shallow water bodies or small lakes (Álvarez-Cobelas et al., 2006; Parcerisas et al., 2012).

Charophytes are a group of aquatic organisms that can be strongly affected by nitrate levels and increased temperatures. They play a structuring role in aquatic ecosystems since they directly and indirectly structure the planktonic and benthic food webs (Rojo et al., 2013, 2017a), and they act as nitrate sinks because the amount of nitrate they take up from the water column is higher than that released by decomposition (Kufel and Kufel, 2002; Rodrigo et al., 2007).

The effects of an increase in nitrate levels on charophytes are not fully understood. Some authors linked a reduction in macrophyte (including charophyte) richness to increases in nitrate concentrations of up to 2 mg $N\text{-}NO_3\ l^{-1}$ (Barker et al., 2008; Lambert and Davy, 2011). Yet, Kipriyanova and Romanov (2013), found charophyte species in aquatic systems in western Siberia with nitrogen concentrations much higher than this threshold. Others (Álvarez-Cobelas et al., 2006; Rodrigo and Alonso-Guillén, 2008) reported the healthy growth of *Chara hispida* and *C. vulgaris* in long-lived meadows in different lakes and ponds affected by the seepage of agricultural run-off in Spain, with nitrate concentrations much higher than 2 mg $N\text{-}NO_3\ l^{-1}$. Moreover, we have observed charophyte growth in nitrate threshold microcosm

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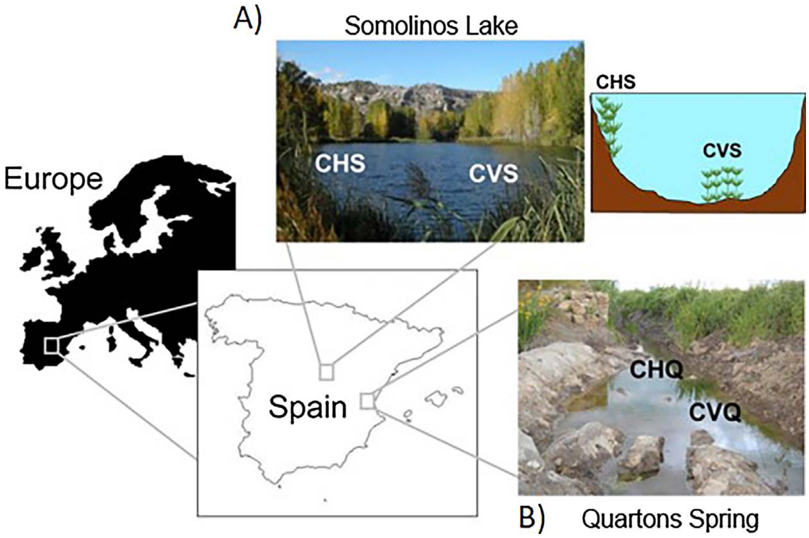

Fig. 1. Location of studied charophyte populations, showing sampling sites: A) the Somolinos mountain Lake and B) the Quartons coastal Spring. There were meadows of *Chara hispida* and *Chara vulgaris* in both sites: CHS and CVS (*C. hispida* and *C. vulgaris* from the Somolinos Lake), CHQ and CVQ (*C. hispida* and *C. vulgaris* from the Quartons Spring).
Source: Miguel Álvarez-Cobelas photographed the mountain lake and Acció Ecologista-Agró took the photograph of the coastal spring, both pictures taken in 2016.

experiments (without microalgae competition) at concentrations ranging from 0.5 to 50 mg N-NO_3 l^{-1} (Rodrigo et al., 2017).

A few studies tested both the direct relationship between the nitrogen concentration in the medium and its uptake and storage by *Chara* spp. and the differences between aboveground and belowground uptake (Vermeer et al., 2003; Rodrigo et al., 2017). Different populations of *Chara vulgaris* responded to temperature changes according to the altitude of their habitat, implying different genetic capacities for adaptation and different reaction norms depending on the local conditions (Rojo et al., 2015). Recently, the interactive and antagonistic effect of warmer temperatures and increases in salinity has been shown for two *Chara* species (Rojo et al., 2017b).

Warmer temperatures led to an increase in the growth and metabolic rates of charophytes, and these increases modified charophyte stoichiometry (Rojo et al., 2015, 2017b). However, it is currently unclear what occurs when more nitrate is available. The novelty of the current study is the analysis of the response of two cosmopolitan charophyte species (*Chara hispida* and *Chara vulgaris*) to sudden and concomitant, but realistic, changes in nitrate concentration and temperature. *Chara hispida* and *C. vulgaris* co-occur in many ecosystems of southern Europe (e.g., Spain; Cirujano et al., 2008). Although both have been described as ‘generalist’ species (Rey-Boissezon and Auderset Joye, 2015), they are not redundant species, as their autecology is somewhat different. *Chara vulgaris* is clearly a pioneer species, as it is the first to germinate from seedbanks. It has great expansion ability, with high fertility and high growth rates (Moore, 1986; Rodrigo et al., 2017), while *C. hispida* has lower growth rates, although it can form dense and monospecific meadows in a wide range of habitats (Barinova et al., 2014; Rojo et al., 2017b). Populations of both species co-occurring in the same ecosystem differ in their response to salinization and increased temperatures, and *C. vulgaris* was shown to have faster growth rates in all the conditions tested (Rojo et al., 2017b). The response of charophytes to changes in environmental conditions depends on the phenotypic plasticity of populations and the existence of ecotypes (Rojo et al., 2015, 2017b). Such differential responses to local environmental variation would result in changes in the diversity of charophyte communities. There are important relationships between charophytes and the abiotic or biotic environment which are species-specific, such as the nutrients incorporation or the allelopathy and its effects over plankton and epiphtic community (Kufel and Kufel, 2002; Rodrigo et al., 2017; Rojo et al., 2013, 2017a). Therefore, the loss of biodiversity, finally, may alter ecosystem functioning (e.g. clear water phase, biogeochemical cycles, carbon sink), with shallow ecosystems being particularly vulnerable to the aforementioned global changes (Auderset Joye and Rey-Booissezon, 2015; Rodrigo et al., 2013; Rojo et al., 2017b). Thus, it is necessary to consider populations originating from different environmental conditions when studying the interactive effect of two factors, such as increases in nitrate concentrations and temperature (Hyldgaard and Brix, 2012; Cross et al., 2015). For this reason, we chose *C. hispida* and *C. vulgaris* populations from two Spanish sites that clearly differ in their nitrate loading, Somolinos mountain Lake and Quartons coastal Spring. In a laboratory experiment, we subjected the four populations to increases in nitrate concentration and temperature that are foreseeable based on current global change predictions: a two-fold increase in nitrate concentration with respect to their habitats of origin and a 4 °C increase in temperature. Our first hypothesis is that the charophyte species will show an increase in growth and/or morphological or physiological changes in response to an increase in nitrate concentration. The second hypothesis is that higher growth rates mediated by warmer temperatures will favour nitrate uptake and that the synergistic effect of temperature and water nitrate concentration can affect charophyte stoichiometry. We expect that the population responses will depend on the phenotypic plasticity of the charophyte species, and might depend on the local conditions of origin.

2. Materials and methods

2.1. Population origin and culture

The specimens of *C. hispida* and *C. vulgaris* used in the experiment were collected from two different sites: Somolinos Lake (Sierra de Ayllón Protected Area, 1270 m a.s.l., 41°15′04″N, 3°03′54″W), an oligotrophic, deep (7 m maximum depth), mountain lake located in a cold climate, and Quartons Spring (Almenara, Castellón, 0 m a.s.l., 39°45′16″N, 0°11′27″W), a eutrophic, shallow (0.6 m maximum depth) water body located in a warmer climate (Fig. 1, Table 1). In Somolinos Lake, *C. hispida* (CHS) grows in a dense meadow in the littoral zone, while *C. vulgaris* (CVS) is located much deeper, close to the lake bottom, forming scattered patches. In Quartons Spring, *C. vulgaris* (CVQ) is the dominant charophyte throughout its extension and forms a dense meadow that almost reaches the water surface. Scattered among this species, *C. hispida* (CHQ) also forms dense patches.

The harvested charophytes were transported to the laboratory at the University of Valencia. The plants were gently washed, and the apical parts with a few nodes were cut and planted in small pots containing a mixture of sand and sediment (2:1 ratio); the sediment used was a 50% mixture of sediment from each place of origin (Table 1). The pots were placed in containers filled with dechlorinated tap water until the charophytes began to grow (Rojo et al., 2015). These stock cultures