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Research article

Sr isotope discrimination of multi species aquaculture productions at a worldwide scale and contribution of the water reservoir in Sr plant input

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A B S T R A C T

Aquatic plants commonly used in landscaping or as ornaments are subject to a growing worldwide market that is source of trade between countries which can induce the transfer of unwanted invasive alien plant species. To protect national biodiversity and economy, authorities promote the use of local markets without however providing the method to do so. This study deals with the feasibility of using Sr stable isotopes for discriminating the origin of aquatic plants at a worldwide scale. More than 15 aquatic plant species were collected from main producers in four countries (China, Hungary, Germany, France). Each plant was analysed for its $^{87}Sr/^{86}Sr$ ratio and the results are compared within samples from each producer and between countries. For a given produce, significant $^{87}Sr/^{86}Sr$ can be measured as a function of the plant species. However, at the scale of Europe, plants from different producers are almost isotopically identical with $^{87}Sr/^{86}Sr$ ratios of 0.71228 ± 218.10^{-5}, 0.71116 ± 178.10^{-5} and 0.71066 ± 156.10^{-5} for France, Hungary and Germany, respectively. These values are clearly distinguishable from those measured in Chinese aquatic plants, which yield a mean $^{87}Sr/^{86}Sr$ ratio of 0.70591 ± 168.10^{-5}. This Sr isotopic discrimination between European and Chinese aquatic plants is explained by the specific agricultural and hydrogeological environments of the producing countries. Indeed, the cycle of Sr in aquatic plants is studied here for the first time. Natural and anthropic materials of French origin were collected and characterized in terms of $^{87}Sr/^{86}Sr$. Mixing models reveal that irrigation water is the main source of Sr for plants (35–85%). Clays from the substrate (4–38%) and fertilizers (5–19%) can also contribute, in proportions depending on the plant species. This model accounts for the small variations in $^{87}Sr/^{86}Sr$ ratios of species from the same producer and allows a discrimination between producers using different agricultural practices.

1. Introduction

Invasive alien plant species (IAPS) are considered as one of the major threats to biodiversity (Dehnen-Schmutz and Touza, 2008; Lambertini et al., 2011; MEA (Millennium Ecosystem Assessment), 2005; Simberloff et al., 2013; Vilà et al., 2011; Young and Larson, 2011). By definition an IAPS is a plant having negative effects on the biosphere (IUCN, 2000). There is nevertheless an ongoing debate mostly due to a confusion between the terms “invasive” and “alien” (Colautti and Macisaac, 2004; Humair et al., 2014; Richardson et al., 2000; Russell and Blackburn, 2017). The expansion and colonization of IAPS has increased with human mobility (Keller et al., 2011), the degree of market openness and the choices of policymakers. For instance, some scientists recommend a *laissez faire* policy and find some benefit from IAPS (Bonanno, 2016; Chapman, 2016; Mugido et al., 2014). They are however in minority because this benefit is limited to a few individuals or companies at the detriment of the population (Tong et al., 2018). The population's perception of IAPS is mostly in favour of their control to protect biodiversity and human well-being (Lindemann-matthies, 2016; Potgieter et al., 2019). This approach is necessary in order to set up effective countermeasures (Fischer and Young, 2007; Olszańska et al., 2016; Perrings et al., 2002) since peoples' perceptions can influence policies on IAPS (Eiswerth et al., 2011). Under pressure, governments enact regulations to prevent the deliberate introduction of IAPS (European Union, 2014; WTO, 1994) and conduct campaigns of control and eradication on their territories to combat this threat (Hussner et al., 2017). The costs generated by the presence of IAPS are difficult to quantify due to indirect effects or invisible effectiveness (Jardine and Sanchirico, 2018), but

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countries colonized by these species spend millions – and even billions in some cases – in efforts to control them (Jardine and Sanchirico, 2018; Pimentel et al., 2005, 2000; Sinden et al., 2004; Xu et al., 2006), but this approach is often futile (Simberloff, 2014). Indeed, according to existing models on the invasion process, alien species need to pass several stages and barriers (Blackburn et al., 2011; Richardson et al., 2000). Hence, the most cost-effective method is to act at the earliest stage during the introduction, by controlling their transport (Burt et al., 2007; Hussner et al., 2017).

Horticulture is the main pathway for the introduction of IAPS (Burt et al., 2007; Dehnen-Schmutz and Touza, 2008; Hussner, 2012; Maki and Galatowitsch, 2004; Reichard and White, 2001; Wells, 1986) and yet actor in this field of activity are extremely sensitive to this issue with emerging voluntary initiatives (Burt et al., 2007). Nevertheless, the accidental introduction of invasive alien aquatic plant species (IAAPS) increases with global trade and anthropochory (Hulme, 2009; Les and Mehrhoff, 1999; Ma and Li, 2002; Maki and Galatowitsch, 2004; MEA (Millennium Ecosystem Assessment), 2005; Shine, 2007). Horticultural commercial orders often involves non-requested species and even prohibited IAPS (Maki and Galatowitsch, 2004). This observation is all the more worrying since the number of IAPS does not need to be high to inflict significant damage to ecosystems (Pimentel et al., 2000).

The position of China on IAAPS is particularly concerning. Its tremendous economic growth has triggered environmental threats from an expanding list of biological invaders (Ding et al., 2008; Ding and Wang, 1998; Hulme, 2009). A total of 265 IAPS have been identified and this number is increasing exponentially over time (Xu et al., 2012). Even if the majority of IAPS were intentionally introduced (67.9 %), a large proportion (31.7 %) appears to have resulted unintentionally (Xu et al., 2012). Shanghai, the largest city of China and third biggest container port in the world (Yang and Nakagoshi, 2004), is not exception, with 57.4 % of its 890 angiosperms being non-native species (Li et al., 2001). In this city, 90 IAS – taking flora and fauna together – have been counted and the number is rising. This is linked to the presence of the harbour, with hulls and ballast water being recognized as vectors of IAS introduction (Elliott, 2003; Hulme, 2009; Mooney, 1999; Wasson et al., 2001).

Nowadays, Asia is one of the main worldwide exporters of aquatic plant species, while France, Germany and Hungary are among the main importers belonging to the European and Mediterranean Plant Protection Organization (EPPO) (Brunel, 2009). Urgent actions need to be taken to achieve the target of the Strategic Plan for Biodiversity (2011–2020) of the Convention on biological Diversity (CBD) (Lambertini et al., 2011). To prevent accidental introduction of IAPS, horticultural producers are wanting to promote local trade in this field due to two major issues (i) from an environmental and phytosanitary point of view, pointing out the risk of spread of diseases or disappearance of endemic species, (ii) from the point of view of human well-being, dealing with the preservation of biodiversity for future generations.

To protect their territories, countries like France advocate the use of native plants (European Union, 2014). Quality labels and geographical indications exist. However, no tool or method is recommended for the recognition and control of the local origin of aquatic plants. However, certain tracers could be used to identify the geographical origin. Monospecific studies have shown the feasibility of using elemental composition (Gonzalvez et al., 2009; Zhao et al., 2013), genetic data (Ali et al., 2014; Huang et al., 2012), or stable isotopes (O, C, H, N and S) (Goitom Asfaha et al., 2011; Liu et al., 2018; Park et al., 2019) to identify the geographical origin of a vegetal or a secondary agrifood product. Each one of these systems has its limits and a multicomponent approach is often required. Among these methods, the analysis of stable Sr isotopes is particularly interesting, and has already been used to control some labels such as AOP or AOC on many agricultural products such as olive oil (Janin et al., 2014; Medini et al., 2015; Portarena et al., 2017; Techer et al., 2017), fruit juice (Rummel et al., 2010), coffee (Rodrigues et al., 2009; Techer et al., 2011), wine (Almeida and Vasconcelos, 2001; Barbaste et al., 2002; Durante et al., 2018; Epova et al., 2019; Lancelot et al., 1999; Vinciguerra et al., 2016) or crops (Choi et al., 2008; Kawasaki et al., 2002; Oda et al., 2002; Song et al., 2014; Voerkelius et al., 2010). The method is based on the biogeological cycle of Sr and the specificity of each growing environment in terms of the $^{87}Sr/^{86}Sr$ isotopic ratio: Sr-cycling in terrestrial plants is well documented and strontium is considered to be taken up principally from the soil (exchangeable phase), which allows the use of the $^{87}Sr/^{86}Sr$ ratio as a tracer of the geographical origin of a plant crop (Åberg et al., 1990; Capo et al., 1998; Graustein, 1989; Stewart et al., 1998). Little is known on the role of a large water reservoir in contributing to the uptake of Sr during plant growth as in the case of aquaculture.

Like calcium, strontium is taken up by plants mainly from the root network in various compartments of the ecosystem, leading to a mixing of Sr in biological tissues (Capo et al., 1998; Downie et al., 1998; Isermann, 1981; Stewart et al., 1998). The isotopic composition $^{87}Sr/^{86}Sr$ of a system depends on the age and nature of the material due to the radiogenic ^{87}Sr arising from the slow radioactive decay of ^{87}Rb. It is commonly accepted that the $^{87}Sr/^{86}Sr$ signature in a plant does not undergo significant fractionation during extraction and translocation (Graustein, 1989). Hence, the measured Sr isotopic ratio reflects mixing in the natural or aquaculture environments (Techer et al., 2017).

Contrary to terrestrial plants, aquatic plants grow in the presence of a large water reservoir, whose imprint on the Sr biogeological cycle is not constrained. However, the water used for growing crops of aquatic plants is dependent on the local situation, been mostly pumped at the production site for economic reasons (groundwaters). Moreover, the plant substrates, amendments and fertilizers used are specific to each producer. Under these conditions, it would appear possible to distinguish the geographical origin of aquatic plants using the $^{87}Sr/^{86}Sr$ tracer because of different geological and hydrogeological media and also the specific aquaculture practices (Techer et al., 2017).

This study aims to characterize, for the first time, the potential use of strontium isotopes to recognize and discriminate the origin of aquaculture produce at a multi-species scale. Our objective is to detect mislabelling practices which correspond to importation of a “foreign” plant and its resale under a false origin. We focus on European and Chinese produce, by considering 15 species commonly encountered in natural wetlands and commonly in demand on the market. The contribution of a large water reservoir to the Sr biogeological cycle is constrained and discussed by studying a French agricultural product (Nymphea Distribution).

2. Materials and methods

2.1. French aquaculture plant production

The Nymphea Distribution company is a leader in the production of aquatic plants in France. The aquaculture practices employed by this company are based on the use of a specific substrate for the first development of plants, which is composed of white/black peat and clay minerals. The detailed composition of this substrate designed by the company is known but kept confidential. Water used for irrigation and flooding by this company is pumped directly from the underlying Vistrenque aquifer (Sassine et al., 2015). Some natural products such as guano and dry poultry blood are occasionally used as fertilizers and pesticides during production and cultivation.

2.2. Plant sampling

Aquatic plants were collected from four main producers in four countries selected from the studied market. Since our aim was to discriminate aquaculture products whatever the plant species, we considered a range of different aquatic plant species from a given producer.

A total of 17 samples belonging to 9 aquatic species were supplied by the company Nymphea Distribution in France (Cailar, 30740).

Species were selected considering their wide use for ecosystemic functions such as *Phragmites australis*, used in sewage treatment or phytopurification of organic pollutants, as well as *Sparganium erectum*, *Cyperus longus* and *Typha latifolia*, which are commonly used in civil engineering and industrial water treatments. Other species were also studied, including *Juncus maritimus, Butomus umbellatus* and *Carex pendula* (Table 1). The studied samples were produced by Nymphea Distribution in 2015 and 2016. Some specimens of these species were also encountered in the natural environment close to the Nymphea Distribution company site (Pont des Tourradons, Cailar, France). Nine samples of these "natural" plants were collected for comparison (Table 1).

Table 1. Studied aquatic plants: species, origin, year of production, and $^{87}Sr/^{86}Sr$ ratios. (SD = standard deviation, 1σ).

Geographical origin	Species	Year of production	$^{87}Sr/^{86}Sr$	SD ($.10^6$)	Production mean	SD ($.10^5$)	$IC_{95\%}$
Hungary	*Butomus umbellatus*	2016	0.710957	4.10	0.71116	89	0.710612–0711723
Hungary	*Butomus umbellatus*	2016	0.710919	2.97			
Hungary	*Scirpus lacustris*	2015	0.710085	4.00			
Hungary	*Scirpus lacustris*	2016	0.712008	7.30			
Hungary	*Scirpus lacustris*	2016	0.711770	3.13			
Hungary	*Sparganium erectum*	2015	0.710979	2.70			
Hungary	*Sparganium erectum*	2015	0.711411	4.22			
Hungary	*Sparganium erectum*	2016	0.709763	2.98			
Hungary	*Sparganium erectum*	2015	0.712543	2.89			
Germany	*Iris pseudacorus*	2015	0.709535	5.00	0.71066	78	0.710048–0.711155
Germany	*Iris pseudacorus*	2015	0.709527	4.90			
Germany	*Juncus effusus*	2015	0.711161	4.56			
Germany	*Juncus effusus*	2015	0.711274	4.61			
Germany	*Juncus effusus*	2015	0.711236	4.35			
Germany	*Lysimachia vulgaris*	2015	0.710939	6.00			
Germany	*Lysimachia vulgaris*	2015	0.710914	4.83			
France	*Carex pendula*	2015	0.711172	2.50	0.71228	109	0.711837–0.712836
France	*Carex pendula*	2015	0.711129	4.28			
France	*Carex pendula*	2015	0.711598	3.99			
France	*Alisma plantago*	2015	0.712248	3.00			
France	*Alisma plantago*	2016	0.715558	3.57			
France	*Baldellia ranunculoides*	2016	0.712484	4.16			
France	*Juncus inflexus*	2016	0.713161	3.24			
France	*Lythrum salicaria*	2016	0.711813	4.08			
France	*Phragmites australis*	2016	0.712250	6.00			
France	*Phragmites australis*	2016	0.712263	3.39			
France	*Phragmites australis*	2016	0.712227	3.92			
France	*Sparganium erectum*	2016	0.713502	4.11			
France	*Sparganium erectum*	2016	0.712596	4.56			
France	*Sparganium erectum*	2016	0.712446	3.02			
France	*Typha latifolia*	2016	0.710956	4.13			
France	*Butomus umbellatus*	2016	0.711700	4.24			
France	*Butomus umbellatus*	2016	0.711660	2.94			
France (natural)	*Cyperus longus*	2015	0.711314	3.00	0.71151	37	0.711283–0.711765
France (natural)	*Cyperus longus*	2015	0.710960	4.77			
France (natural)	*Cyperus longus*	2015	0.711326	5.49			
France (natural)	*Cyperus longus*	2015	0.711426	3.60			
France (natural)	*Cyperus longus*	2015	0.711436	4.75			
France (natural)	*Cyperus longus*	2015	0.711463	5.72			
France (natural)	*Juncus maritimus*	2015	0.712094	7.00			
France (natural)	*Juncus maritimus*	2015	0.711484	3.91			
France (natural)	*Juncus maritimus*	2015	0.712126	14.8			
China	*Cyperus longus*	2016	0.704648	3.49	0.70591	84	0.705404–0.706391
China	*Cyperus longus*	2016	0.704658	3.29			
China	*Iris pseudacorus*	2016	0.706686	4.23			
China	*Phragmites australis*	2016	0.705627	3.74			
China	*Phragmites australis*	2016	0.705677	8.95			
China	*Butomus umbellatus*	2016	0.705337	3.89			
China	*Scirpus lacustris*	2016	0.706453	3.99			
China	*Sparganium erectum*	2016	0.706711	3.82			
China	*Sparganium erectum*	2016	0.706686	2.21			
China	*Typha latifolia*	2016	0.706637	3.18			

At the scale of Europe, we obtained a number of species produced by a Hungarian and a German producer: 9 plant samples of *Butomus umbellatus*, *Scirpus lacustris* and *Sparganium erectum* were collected from Hungary (Kecskemet, 6000), and 7 plant samples of *Iris pseudacorus, Juncus effusus* and *Lysimachia vulgaris* from Germany (Schwarmstedt, 29685).

Hence, to study production from outside Europe, we considered 10 specimens of the same species as those studied in Europe, but imported from Shanghai (China, 200000) (Table 1). Aquaculture plant products from China were selected due to the peculiar position of China in global trade as one of the main worldwide exporters of aquatic plants.

In summary, the present study covers a total of 52 plants (n = 52) including 15 species produced in 2015 and 2016 in Europe (France, Le Cailar, n = 17; Hungary, Kecskemet, n = 9; and Germany, Schwarmstedt, n = 7). We also studied seven of these species produced during 2016 in China (Shanghai, n = 10), and, for comparison, we included two species (n = 9) collected in 2015 from a natural environment near the French producer.

2.3. Methods and analyses

2.3.1. Plants

For a given producer and species, the aerial parts of the plant were cut, dried at 75 °C for at least 48 h (Air performance stove, Froilabo) and then ground with a ball mill (SPEX 8000, SPEX Industries Inc). A powder sample of 250 mg was then digested in a solution of 8 ml 7 N HNO_3 and 2 ml of 1 N H_2O_2 of high-purity grade in a high-temperature closed-vessel microwaved-assisted reactor (ETHOS One, Milestone). The recovered solution was then evaporated on a hotplate at 60 °C in a PTFE beaker.

2.3.2. Substratum, water and cultural products

Different components of the French aquaculture production environment were sampled: irrigation water (n = 2) and substrate (n = 3) which was composed of clay (n = 2), white peat (n = 1) and black peat (n = 1). Fertilizers such as poultry dry blood (n = 2) and guano (n = 2) were also sampled. The bioavailable phase of these compounds was analysed in each case. For this purpose, samples were dried at 105 °C for at least 48 h (Air performance stove, Froilabo) and ground with a ball mill (SPEX 8000, SPEX Industries Inc). The exchangeable phase of the obtained powder was extracted following Takeda et al.'s procedure (Takeda et al., 2006). 200 mg of each sample was weighed and then washed with 4 ml of 1 M NH_4NO_3 for 2 h under rotating agitation (Reax 20/4, Heidolph) at 16 rpm. Samples were then centrifuged (Centrifuge 5804, Eppendorf) at 8500 rpm for 10 min. Supernatant was collected and dried. A 2 ml aliquot of the irrigation water was evaporated for Sr treatment.

2.3.3. Sr contents

Dry residues were taken up with 500 µl of 0.05 N HNO_3 and 9.5 ml of H_2O milliQ then analysed by ionic chromatography (930 Compact IC Flex, Methrom) with a Metrosep C6 – 150/4.0 column and using 2,6-Pyridinedicarboxylic acid as an eluent. The effectiveness of the analytical method was tested by cross analysis with ICP-MS (Neptune, Thermo Scientific, analyses performed by the CNRS/UdS, France).

2.3.4. $^{87}Sr/^{86}Sr$ composition

Sr elemental separation was carried out according to the protocol of Pin et al. (Pin et al. (2003) on Sr-resin (EICHROM). The dry residue was taken up with 0.5 ml of 2 N HNO_3 and then introduced by adding 100 µl to a PFA column containing 33 mg of Sr Spec sps EXC resin pre-cleaned with 1 ml of 6 N HCl, 1 ml of HNO_3 0.05 N and reconditioned with 2 × 100µl of 2 N HNO_3. Elution of the elements other than Sr was carried out by successive passages of 4 × 100 µl of 2 N HNO_3 then 1 ml of 7 N HNO_3 and 200 µl of 2 N HNO_3. The strontium was then recovered by eluting 1 ml of 0.05 N HNO_3. The recovered Sr solution was then evaporated in a PTFE beaker on a hot plate at 60 °C.

The dry Sr taken up in 1 N HNO3 was deposited on a tantalum filament for analysis by Thermal Ionization Mass Spectrometry (TIMS) (TRITON, ThermoFinnigan). The measurement takes account of internal corrections with respect to the stable $^{86}Sr/^{88}Sr$ ratio and also the presence of rubidium (Rb) which interferes with mass peak 87. The analysis of a sample corresponds to a succession of blocks of 15 measurements of the different isotopic ratios; the number of blocks is variable according to the intensity of the recorded signal and thus the type of sample. On average, 5 blocks (75 measurements) are carried out for the plants. Under these conditions, the error obtained on the measurement, expressed in 2σ, is estimated at between 6.10^{-6} and 20.10^{-6}. The accuracy of the measurement is determined by the analysis of a standard, NBS 987, whose theoretical isotopic ratio is 0.71034 ± 26.10^{-5} (2σ) with an "accepted value" of 0.710263 ± 16.10^{-6} (2σ) (Stein et al., 1997). The repeated analysis of this sample yields an average of $0.710265 \pm 16 .10^{-6}$ (2σ, n = 25).

2.4. Statistical analysis

The statistical analyses were carried out with the software R (R Core Team, 2018) (version 3.5.1) and the R studio environment (RStudio Team, 2016) (version 1.1.456). The normality of the distributions was tested with the Shapiro-Wilk test. Differences between populations were tested with Student's t test for populations with normal distributions and homogeneous variability distributions and the Kruskal-Wallis test for non-parametric distributions using the *stats* package (R Core Team, 2018). Confidence intervals at 95 % around mean values were calculated through 1,000 bootstrap replications with the *boot* package (Canty and Ripley, 2017; Davison and Hinkley, 1997). Mixing calculations were carried out using the *simmr* package based on Bayesian statistics with Markov chain Monte Carlo methods performed with 100,000 iterations and 100 blocks (Parnell, 2016). Calculations were performed for each plants from the French producer and the results were merged to investigate correlations and then analysed with a matrix plot function adapted to show bivariate density distributions.

3. Results

3.1. Sr isotopic composition of plants and discrimination of producers

Aquatic plants produced by Nymphea Distribution in the South of France are characterized by $^{87}Sr/^{86}Sr$ isotopic ratios ranging between 0.710956 ± 8.10^{-6} (2σ) and 0.713502 ± 8.10^{-6} (2σ) with an outlier at 0.715558 ± 8.10^{-6} (2σ). Native plants from the natural environment near this French site (within 10 km) yield similar ratios ranging from 0.710960 ± 10.10^{-6} (2σ) to 0.712126 ± 30.10^{-6} (2σ). Plants from Hungarian and German producers are respectively identified by $^{87}Sr/^{86}Sr$ with ranges of 0.709763 ± 6.10^{-6} (2σ) - 0.712543 ± 6.10^{-6} (2σ) and 0.709527 ± 10.10^{-6} (2σ) - 0.711274 ± 10.10^{-6} (2σ). Chinese aquatic plants have distinguishable $^{87}Sr/^{86}Sr$ ratio ranging from 0.704648 ± 9.10^{-6} (2σ) to 0.706711 ± 8.10^{-6} (2σ) (Table 1).

For the same producer, variations of Sr isotopic ratio are observed according to the year of production (known for France) and the species concerned. A statistical analysis helps us to detect potential year- or species-bias for a mean composition of a plant production. The isotopic ratios of German (Shapiro-Wilk, p = 0.009), French (Shapiro-Wilk, p = 0.011) and Chinese (Shapiro-Wilk, p = 0.001) plant productions do not show a normal distribution. The Hungarian production (Shapiro-Wilk, p = 0.886) and plants from the French natural environment (Shapiro-Wilk, p = 0.084) show a normal distribution of $^{87}Sr/^{86}Sr$ ratios. However, in view of the densities of each population (Figure 1), we can assume that the small size of the two latter populations impacts the result of the statistical test and therefore it is quite difficult to assess the normality of these distributions. To maintain consistency of results, all the distributions in this study are considered as non-normal. In this way, a statistically significant difference is revealed in the Sr isotope composition of

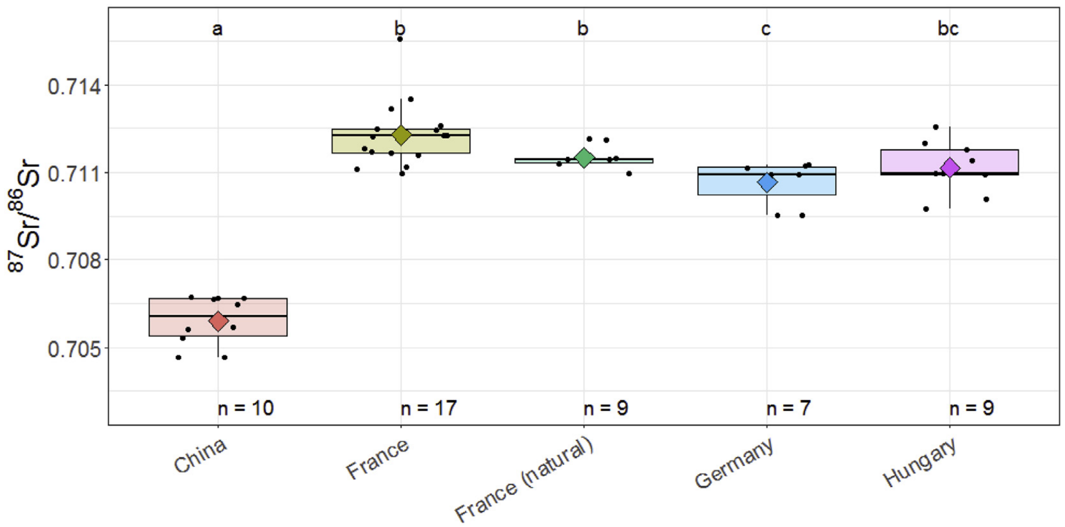

Figure 1. $^{87}Sr/^{86}Sr$ ratios of plants as a function of their geographical production. Distribution of isotopic $^{87}Sr/^{86}Sr$ ratios of each individual plant for each producer. Black dots represent individual values of $^{87}Sr/^{86}Sr$ of plants, while grey diamonds correspond to the mean $^{87}Sr/^{86}Sr$ value for the total production (error bars representing standard deviation on the $^{87}Sr/^{86}Sr$ of individual plant values are not visible at the scale of the diagram).

French plants (Wilcoxon test, p = 0.048) when considering the year of production, i.e. a mean $^{87}Sr/^{86}Sr$ ratio of 0.71154 ± 52.10^{-5} (median = 0.71139, $IC_{95\%}$ = 0.71103–0.71205) is obtained in 2015, and 0.71251 ± 113.10^{-5} in 2016 (median = 0.71226, $IC_{95\%}$ = 0.71190–0.71312) (Figure 2). Considering this same population, a difference in Sr composition is also observed as a function of the studied species (Kruskal test, p = 0.037) (Figure 3). This conclusion is also applicable to natural French plants (Kruskal test, p = 0.020) with, for example, *Juncus maritimus* showing higher $^{87}Sr/^{86}Sr$ values than *Carex pendula*, yielding values, respectively, of 0.71190 ± 36.10^{-5} (median = 0.71209, $IC_{95\%}$ = 0.71149–0.71231) and 0.71132 ± 19.10^{-5} (median = 0.71138, $IC_{95\%}$ = 0.71117–0.71147). By contrast, no species-bias is detected among Hungarian plants (Kruskal test, p = 0.667) (Figure 3). For the plants of Chinese and German origin, insufficient numbers of individuals are available to perform the statistical tests required to detect species-bias.

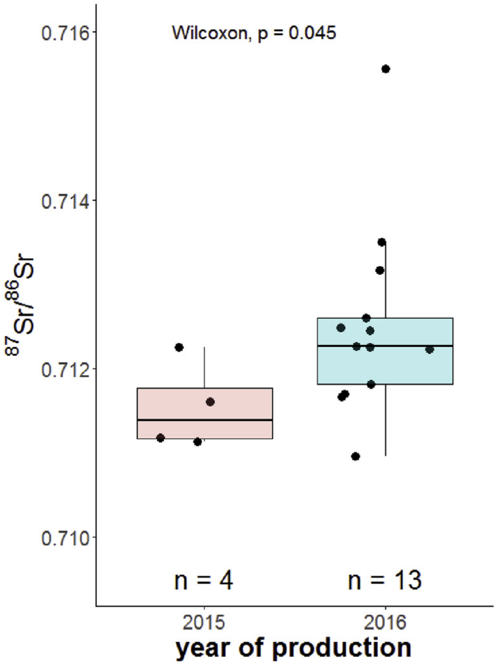

Figure 2. $^{87}Sr/^{86}Sr$ ratios of plants from the French producer according to the year of production (2015 and 2016). Error bars on individual values are not visible at the scale of this diagram.

All species considered, a mean Sr isotopic composition can be determined for each producer. Among European countries, France has the most radiogenic signature with an average ratio of 0.71228 ± 218.10^{-5} (2σ). Hungary and Germany produce plants with lower Sr isotopic ratios, respectively of 0.71116 ± 178.10^{-5} (2σ) and 0.71066 ± 156.10^{-5} (2σ). The three European productions are identified by an average isotopic ratio of 0.71163 ± 236.10^{-5} (2σ). This average value, and the isotopic signature for each European country considered individually, is significantly different from the ratio of 0.70591 ± 168.10^{-5} (2σ) obtained from aquatic plants of Chinese origin.

3.2. *Sr biogeochemical cycle in aquatic plants production*

To constrain the Sr biogeochemical cycle, by considering the sources of Sr taken up by an aquatic plant in the framework of its development in an industrial production process, we study here the case of the French aquaculture company Nymphea Distribution. The aquaculture production process is specific to each producer, since this involves the plant substrate and flood irrigation water used by the producer, with water being commonly pumped from a well belonging to the company (groundwater), while the substrate is made up by the producer with different fertilizers and pesticides being added in varying proportions. All these natural and anthropic materials are characterized by their Sr isotopic signature in the immediate environment of the Nymphea Distribution company. The Sr elementary and isotopic composition of the plants is compared to those of the aquaculture environment (water, substrate and its various constituents, fertilizers and pesticides) (Table 2).

The plant substrate is characterized by an average isotopic ratio of $^{87}Sr/^{86}Sr$ of 0.714054 ± 224.10^{-6} (2σ, n = 3) and a Sr content at 4.8 ± 1.6 μg g^{-1} (2σ, n = 2). In this substrate, the clay and black peat contain radiogenic Sr with isotopic ratios of 0.716805 ± 18.10^{-6} (2σ) and 0.715824 ± 10.10^{-6} (2σ), respectively. However, these constituents are Sr-poor with average contents of 9.0 ± 2.2 (2σ, n = 2) and 5.5 ± 0.4 μg g^{-1} (2σ, n = 2), respectively. Other constituents of the substrate, such as dry poultry blood and guano, are less Sr radiogenic with isotopic ratios, respectively, of 0.708880 ± 18.10^{-6} (2σ), 0.709874 ± 2.10^{-3} (2σ, n = 2), while being poor in Sr with contents of 8.3 ± 1.6 (2σ) and 19.2 ± 2.4 μg g^{-1} (2σ). The irrigation water is characterized by a Sr content of 480 ±

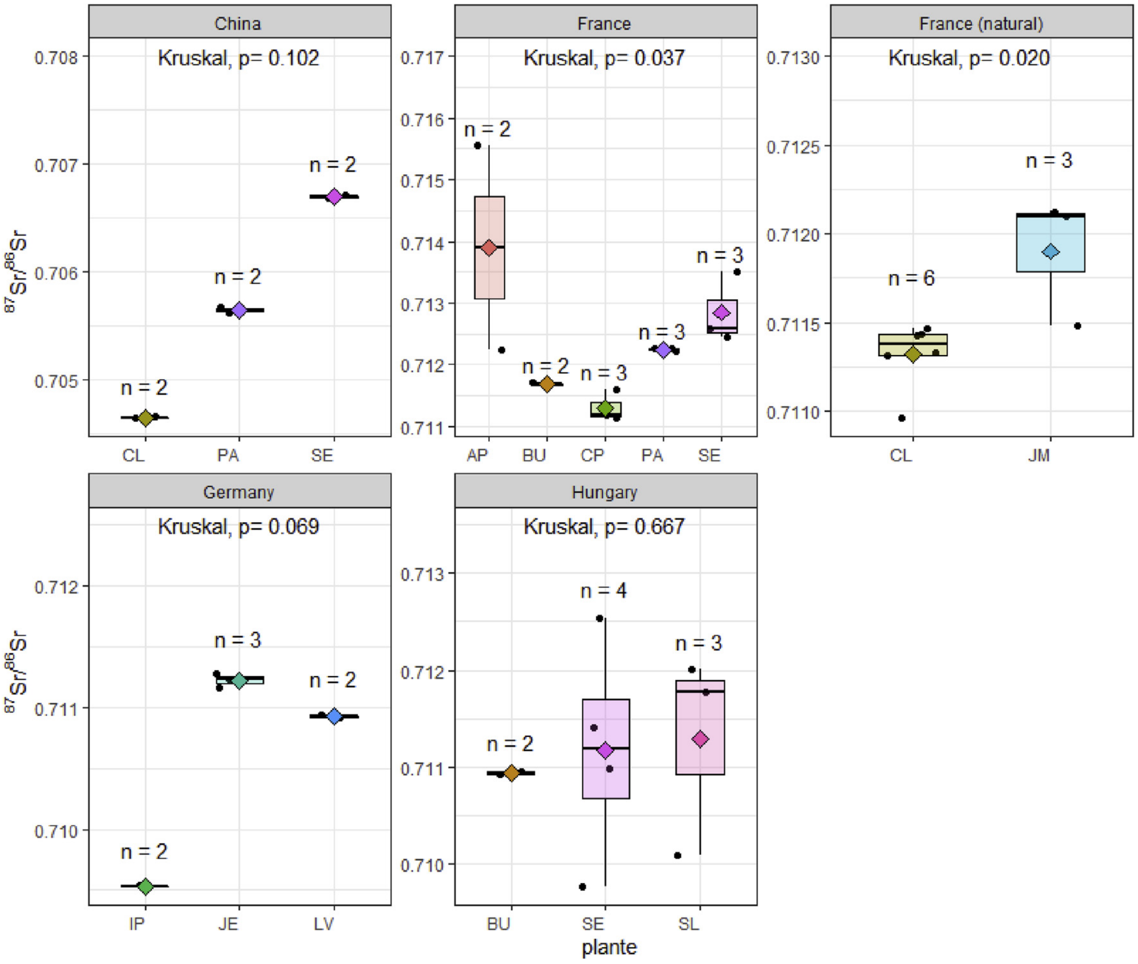

Figure 3. **$^{87}Sr/^{86}Sr$ ratios of plants according to the species for each geographical production**. Black dots represent individual values of $^{87}Sr/^{86}Sr$ of plants while grey diamonds correspond to the plant's mean $^{87}Sr/^{86}Sr$ value. Error bars representing standard deviation on the $^{87}Sr/^{86}Sr$ of individual plant values are not visible at the scale of the diagram. (AP = *Alisma plantago*, BU = *Butomus umbellatus*, CL = *Cyperus longus*, CP = *Carex pendula*, IP = *Iris pseudacorus*, JE = *Juncus effusus*, JM = *Juncus maritimus*, LV = *Lysimachia vulgaris*, PA = *Phragmites australis*, SE = *Sparganium erectum*, SL = *Scirpus lacustris*). Significance distinction between plants' species was tested using Kruskal Wallis test.

8 μg.L^{-1} (2σ) (cf. 961 ± 35.4 μg g^{-1} (2σ) for the dry residue) and an isotopic ratio of 0.708174 ± 12.10^{-6} (2σ).

The plotting of these data points on a mixing diagram $^{87}Sr/^{86}Sr = f(1/[Sr])$ (Faure, 1986) suggests a complex origin of Sr in the sampled French aquatic plants (Figure 4): the Sr in these plants appears to be the result of mixing between Sr taken up from the flood irrigation water and Sr from some substrate constituents. No direct correlation can be observed between "substrate whole matrix" and water. The contribution of the different potential constituents of the substrate is calculated for each studied plant (Table 3). The water used for flood irrigation represents the most important contribution to Sr input, providing about 70 ± 18% of the Sr in plants (median = 71 %, $IC_{95\%}$ = 55 %–85 %). The remaining part is represented by Sr derived from the clay (14 ± 12% (median = 12 %, $IC_{95\%}$ = 4 %–24 %)) and from the black peat (5 ± 2% (median = 4 %, $IC_{95\%}$ = 3 %–6 %)). Fertilizers also play a role in the mixing, contributing 11 ± 5% (median = 10 %, $IC_{95\%}$ = 7 %–15 %) of the Sr in plants (5 ± 2% (median = 4 %, $IC_{95\%}$ = 3 %–6 %) from dry poultry blood and 7 ± 3% (median = 6 %, $IC_{95\%}$ = 4 %–9 %) from the guano).

Three distinctive groups of aquatic plants can be distinguished:

1) *Juncus inflexus,* showing more affinity for Sr coming from the substrate (46 %) and fertilizers (19 %) than from irrigation water (35 %).
2) *Carex pendula* and *Sparganium erectum* take up their Sr mainly from irrigation water (61–62 %) but also from the substrate (23–27 %) and fertilizers (11–16 %).
3) *Baldellia ranunculoides*, *Butomus umbellatus*, *Phragmites australis* and *Typha latifolia* favour Sr from water, with almost no Sr coming from the substrate (6–11 %) or fertilizers (6–9 %) (Table 3).

The contribution of each Sr-pool depends on the plant species, whereas the contribution from the black peat is almost constant at between 2 % and 8 %. Furthermore, we also find evidence of correlations between sources (Figure 5). Highly negative Kendall's tau coefficients (τ) reflect strong negative correlations between the Sr in flood irrigation water and clay ($\tau = -0.87$, $p < 0.001$), and also between irrigation water and fertilizers ($\tau = -0.66$, $p < 0.001$), highlighting the two most important competing sources of Sr: irrigation water or solid phases (soil and fertilizers). Plants having an affinity with the soil also take up greater amount of strontium from the fertilizers ($\tau = 0.26$, $p < 0.001$).

4. Discussion

The feasibility of discriminating the origin of aquatic plants based on their Sr isotopic composition is demonstrated by considering European and Chinese aquaculture productions. Despite a local species-bias among aquatic plants, the mean Sr isotopic compositions of aquatic plants from European producers appear to be significantly different from Chinese producers. Even though internal heterogeneities are measured among samples from the same producer depending on the year of production or species, Sr isotopes seem to be a good tool to discriminate European from

Table 2. $^{87}Sr/^{86}Sr$ ratios and Sr contents of irrigation water, substrate (whole matrix and its separated compounds), fertilizers and plants collected from the Nymphea Distribution French producer (SD = Standard deviation, 1σ).

Cultural environment				
Compartment	**$^{87}Sr/^{86}Sr$**	**SD (.10^6)**	**[Sr^{2+}] (ppm)**	**SD (ppm)**
Substrate (whole matrix)	0.714128	5.1	4.2	0.8
Substrate (whole matrix)	0.713925	4.5	5.4	1.6
Substrate (whole matrix)	0.714109	4.0	*NA*	*NA*
Clay	0.716805	9.5	9.8	0.4
Clay	*NA*	*NA*	8.2	3.5
Black peat	0.715824	4.8	5.6	1.2
Black peat	*NA*	*NA*	5.3	1.4
White peat	0.711416	5.1	1.6	0.5
White peat	0.711429	23.8	2.9	0.7
Irrigation water	0.708886	3.2	948	174
Irrigation water	0.708873	3.7	973	126
Dry poultry blood	0.709141	18.0	7.7	0.7
Dry poultry blood	0.710607	15.8	8.9	1.7
Guano	0.708174	6.0	18.3	0.2
Guano	*NA*	*NA*	20.0	2.4
Plants				
Name	**$^{87}Sr/^{86}Sr$**	**SD (.10^6)**	**[Sr^{2+}] (ppm)**	**SD (ppm)**
Carex pendula	0.711172	2.6	23.9	8.5
Carex pendula	0.711598	4.0	24.7	5.5
Carex pendula	0.711129	4.3	*NA*	*NA*
Baldellia ranunculoides	0.712484	4.2	71.2	8.5
Baldellia ranunculoides	*NA*	*NA*	74.2	2.2
Butomus umbellatus	0.711700	4.2	56.6	2.3
Butomus umbellatus	*NA*	*NA*	58.4	11.6
Phragmites australis	0.712250	6.0	45.0	4.1
Sparganium erectum	0.713502	4.1	24.7	23.5
Typha latifolia	0.710956	4.1	67.1	5.9
Typha latifolia	*NA*	*NA*	68.8	2.6
Juncus inflexus	0.713161	3.2	14.9	4.2

Table 3. Part of Sr taken up from irrigation water, clay, black pit, poultry, and guano or fertilizers (including guano and dry poultry blood) by aquatic plants during their growing in the context of the French production. Plant species are given with their initials (CP = *Carex pendula*, BR = *Baldellia ranunculoides*, BU = *Butomus umbellatus*, PA = *Phragmites australis*, SE = *Sparganium erectum*, TL = *Typha latifolia*, JI = *Juncus inflexus*). Values are rounded to unity.

Species	%Sr from irrigation water	%Sr from clay	%Sr from black peat	%Sr from poultry	%Sr from guano	%Sr from fertilizers
CP-1	62	16	7	7	9	16
CP-2	62	17	6	6	9	15
BR	88	4	2	2	4	6
BU	85	5	3	3	4	8
PA	80	7	4	4	5	9
SE	61	22	5	5	7	11
TL	87	4	2	3	4	7
JI	35	38	8	8	11	19
Mean (%)	70	14	5	5	7	11
SD (%)	18	12	2	2	3	5
Median (%)	71	12	4	4	6	10
$IC_{95\%}$ (%)	55–85	4–24	3–6	3–6	4–9	7–15

Chinese origins. Thus, this approach could be used to control imports from China, for instance, towards France, Hungary and Germany. To strengthen use of this approach and explain the variation among samples from a given country and/or between producers, this study attempts to determine the origin of Sr in aquatic plants. This approach involves understanding the Sr compositions of aquatic plants. In fact, while the Sr biogeochemical cycle is well known for terrestrial plants with a major contribution of Sr coming from soil minerals, as well as the impact of deposits on leaves and fertilizers in agriculture (Stewart et al., 1998), very little is known about aqueous media. The occurrence of a large water reservoirs in aquaculture is expected to impact the biogeochemical cycle of Sr, which explains the need to place constraints to improve our understanding of the ecological aspects (Burger and Lichtscheidl, 2019). The Sr biogeochemical cycle in aquatic plants is studied here for the first time, based on the global characterization of Sr isotopic compositions in plants produced by aquaculture. This approach shows the contribution of

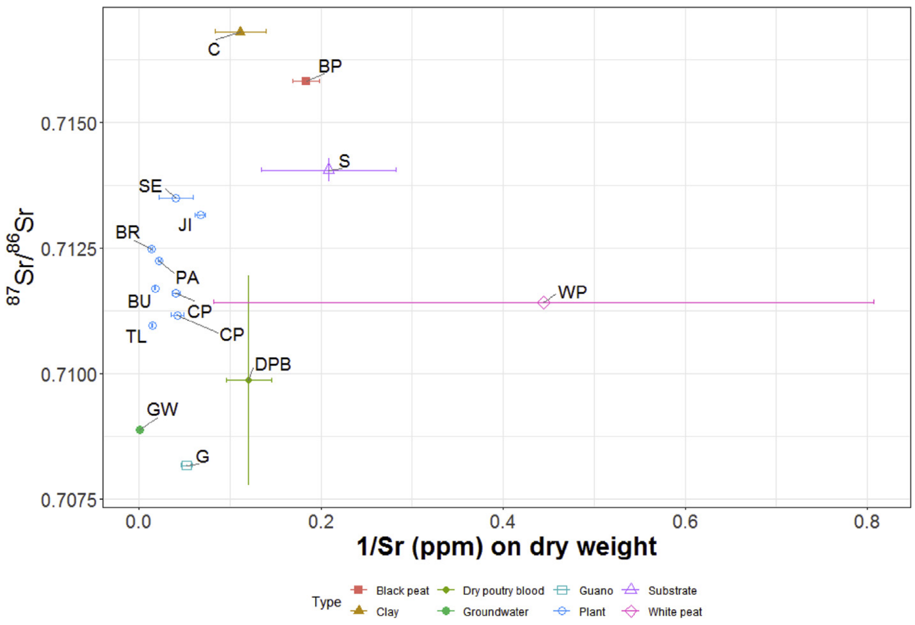

Figure 4. Mixing diagram $^{87}Sr/^{86}Sr$ as function of 1/[Sr] with plants and cultural compounds, data obtained for the French aquatic plant production (Nymphea Distribution). Plant species are referenced by their initials (BR = *Baldellia ranunculoides*, BU = *Butomus umbellatus*, CP = *Carex pendula*, JI = *Juncus inflexus*, PA = *Phragmites australis*, SE = *Sparganium erectum*, TL = *Typha latifolia*). Both ratios and concentrations error bars are 2σ. Individual plant's values are clearly on a line between the irrigation water (dry residue) and the clay underlying a clear mixing between these two sources. Black peat, dry poultry blood and guano may also contribute and be responsible for slight variations around the line. Conversely the white peat is not expected to be involved in the Sr plant uptake.

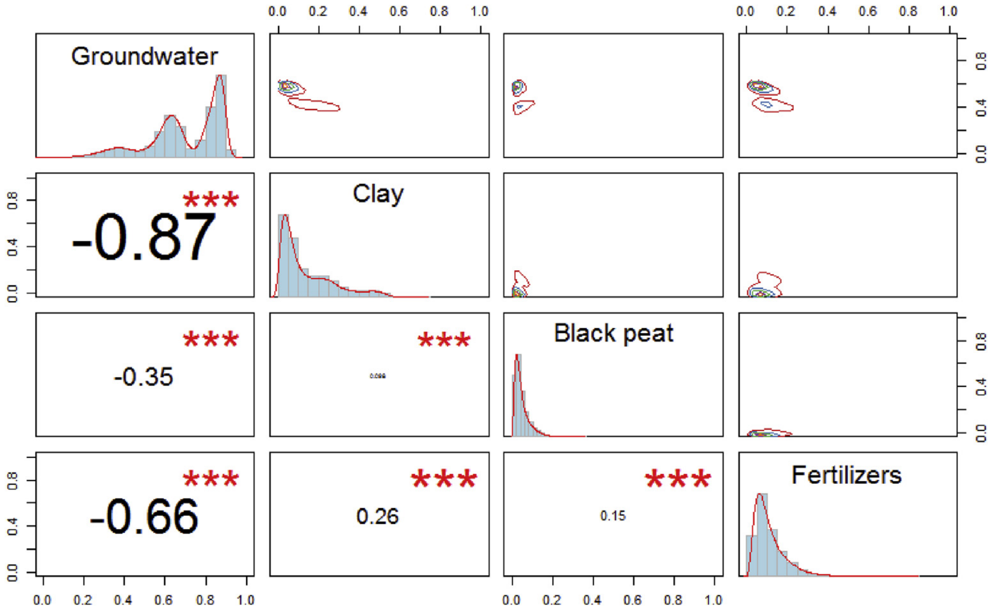

Figure 5. Matrix plot correlation between the identified sources of plant Sr-uptake in the case of the French production. The diagonal of the matrix points out the distribution part of each sources between 0 (0.0) and 100 % (1.0). The lower triangular matrix shows the Kendall correlation plus significance level (as stars). Each significance level is associated to a symbol: p-values 0.001 (***), 0.01 (**), 0.05 (*). The upper triangular matrix is composed by the bivariate density plots. The black peat and fertilizers' distributions are quite homogenous compared to the irrigation water and clay's ones. These lasts sources appear to be species dependent with three different proportion's levels.

various sources to the input of Sr: the water reservoir, the substrate clays and fertilizers. The relative contribution of each source depends on the plant species. Three groups can be distinguished: the first includes plants taking up Sr mainly from the solid phases (substrate and fertilizers), a second group includes plants taking up Sr both from the solid phases and from the irrigation water and a third group corresponds to plants taking up Sr almost exclusively from the irrigation water. Nevertheless, for all the three groups the irrigation water represents a non-negligible source of Sr, with at least 35 % of Sr in plants in the first group coming from this source and up to 88 % in the third group.

In the context of the aqueous media used in aquaculture production, the contribution of various reservoirs to the Sr input in plants helps us explain the $^{87}Sr/^{86}Sr$ ratios measured among samples from the same producer as well as between different producers (Figure 6). Each producer uses a specific substrate composition – often kept secret - which is the most suitable for purpose, and which is formulated with natural rocks and resources of the nearby environment. Moreover, for economic reasons, the irrigation water is usually local water that is in Sr isotopic equilibrium with the geological substratum and thus specific to the geographical setting. Hence, each producer uses fertilizers that are chosen to adapt to fluctuations in climate and production. Irrigation waters from the studied European areas are almost identical in Sr isotopic composition (Voerkelius et al., 2010). The similitude of geological patterns and aquaculture processes in Europe can explain the almost identical $^{87}Sr/^{86}Sr$ ratios of plants produced by aquaculture in Europe. Slight differences between producers are the result of specific practices, for

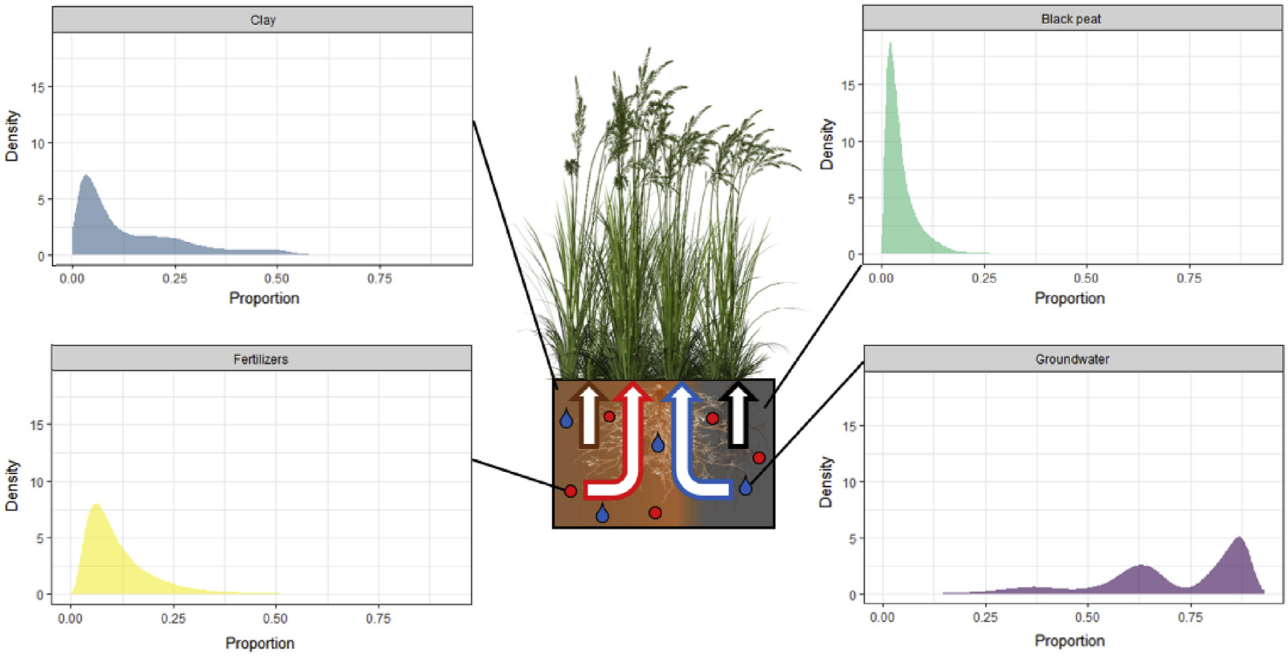

Figure 6. Conceptual scheme of Sr uptake by aquatic plants. Graphics on the left and right sides of this conceptual scheme represent the density proportion of each source involved in Sr cycling in the French production case. Several peaks in these graphics are explained by potential variations in plant metabolism according to species.

instance, due to the addition of fertilizers or the exact composition of the aquaculture substrate. In the same way, this gives an indication of any time-bias.

Sr isotopes appear to be a good tool to discriminate aquatic plants produced by countries characterized by distinct hydrogeological contexts, or coming from countries applying different aquaculture processes. For instance, it helps us to distinguish Chinese from European aquaculture production, and could be used to control importation. No systematic or easily applied discrimination can be made among the three European countries included in this study. However, for the studied market, there is no need of discrimination between countries of the European community. The most severe demands are mainly observed between Europe and foreign producers, such as China. Even if this approach needs to be strengthened with a larger database for the countries studied here as well as other European countries, our study appears promising for the discrimination of aquaculture products in the framework of a stretched market.

5. Conclusion

This study is the first to use strontium stable isotopes as a tracer of the production of aquatic plants, since this needs to be controlled in the framework of a stretched market. Considering various aquatic plant species, Chinese aquaculture products appear to be easily discriminated from European products despite variations among individual producers according to the year of production and species. These variations are explained by the contribution of each reservoir of the aquaculture environment to Sr input, with the major role of large water reservoirs highlighted here for the first time. Different aquaculture processes and environments lead to a range of characteristic $^{87}Sr/^{86}Sr$ isotopic ratios in aquatic species, reflecting the large contribution of Sr from flood irrigation water and from the clay used in plant substrates. This study also shows that adjuvants such as fertilizers can significantly modify the $^{87}Sr/^{86}Sr$ ratio of a cultivated plant. The use of Sr isotopes as a tool represents a competitive tracer for long-distance comparisons, as in the case between China and Western Europe, but has some limits for nearby producers. Furthermore, the time-stability of $^{87}Sr/^{86}Sr$ remains uncertain, and further research is required to study the effect of a sudden change in the aquaculture process. To consolidate these results, a wider study is envisaged with numerous producers around the globe and a greater number of plants species.

Declarations

Author contribution statement

Emmanuel Delattre: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Isabelle Techer: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Benjamin Reneaud: Performed the experiments.

Véronique Thireau: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Patrick Verdoux: Contributed reagents, materials, analysis tools or data.

Philippe Prohin: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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