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**Impact of agricultural practice on the Sr isotopic composition of food products: Application to discriminate the geographic origin of olives and olive oil.**

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**Key words:** bioavailability; strontium uptake; strontium isotope tracing; olive geographic discrimination; agricultural practice.

## Abstract

The Sr isotopic compositions ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) of varied organs (branches, leaves and olives) of olives trees (*Olea europaea*) and those of their growing environment (soils, waters, agricultural products) were determined in two distinct agricultural contexts to discuss the origin of Sr as a function of the irrigation and fertilization techniques. The two studied sites belong to the same geographic area and the same geological basement and soils, but were by subjected to different agricultural practices in terms of irrigation and supply of fertilizers and protection products. The conventional and biological agriculture modalities were defined and tested. Homogeneous  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were measured in the various organs of the trees from a given site. However, the composition was significantly different at the two studied sites. When considering a biological agricultural modality, significant correlations are obtained between the Sr isotopic composition of the tree organs, the soils and the irrigation waters. The mobile and exchangeable fractions of the soils have identical  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios that were transferred to the trees. In case of a conventional agriculture with more intensive irrigation and spreading of plant-protection products, a clear impact of these products is visible in the soils. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the olive trees not only derives from soil sources, but also from added products with a clear role of the irrigation. A slight but noticeable foliar uptake of anthropogenic Sr is also observed. The disturbance of the soil and tree Sr isotope composition as a function of the agricultural practices is discussed in the context of using the Sr isotopic tool as a tracer of the geographic origin of olives and olive oil.

## Introduction

The bioavailability of any element can be defined by the fraction able to be incorporated into a biological reservoir, and can subsequently be used for normal physiological functions (Fairweather-Tait and Hurrell, 1996). In fact, the bioavailability of an element for a given organism is quite complex, hardly defined as directly linked to intrinsic and extrinsic values such as the element solubility, water content, soil mineralogical and chemical composition, temperature, pH and oxidation-reduction conditions, foliage density, rooting depths... (Marschner, 1995; Hinsinger, 1998, 2001; Fuhrmann and Lanzirotti, 2005). However, the origin and intrinsic properties of some chemical elements are of interest for determining the geographical origin of plants in terms of quality and/or authenticity certifications. In this context, the strontium (Sr) content and isotope composition ( $^{87}\text{Sr}/^{86}\text{Sr}$  ratio) of a plant represents an appropriate tool to discriminate agri-food products (Almeida and Vasconcelos, 2001; Barbaste et al., 2002; Fortunato et al., 2004; Swoboda et al., 2008; Techer et al., 2011; Voerkelius et al., 2010; Durante et al., 2013; Hartman and Richard, 2014; Vinciguerra et al., 2015; Petrini et al., 2015). However, as for other elements, the Sr bioavailability is not a single function and numerous internal and external parameters seem to control the capacity of a plant to uptake this trace element and, thus, endorse its source(s). In order to define best the feasibility of using Sr isotopes as a performing tool to discriminate the source of an agri-food product, it appears essential to define the Sr bioavailable reservoirs in precise

agricultural conditions, and to determine which one is most impacting the Sr isotopic composition imprinted in the plant and in its consumable products.

This study aims to investigate the origin of Sr in olive trees (*Olea europaea*) by considering different agricultural practices. It has been undertaken in the frame of a global approach dealing with the feasibility of a geographic identification and recognition of the origin of olive oil (Janin et al., 2014; Medini et al., 2015). The feasibility of Sr isotope measurements in oil was demonstrated by Medini et al. (2015), however a better knowledge of the Sr bio-geochemical cycle during olive trees growth is now expected to consolidate the authentication of olive oil by strontium isotopic tracing. The Sr isotope composition of various parts of olive trees (*Olea europaea*) such as the branches, leaves and olives from two different parcels was compared to those of the interfering environmental components such as the soils, surface water, rainwater. Varied cultivation modalities were also evaluated in the two study cases as functions of the irrigation degree and fertilization technique (conventional or biologic). Two soils fractions, the exchangeable and mobile fractions of the soils that will be defined further down were analyzed to discuss the origin of the Sr taken up by the trees in the different cultivation contexts.

## **1 Sr in plants – The state of the art**

Sr is not an essential element for plants, but it behaves very closely to calcium that is a fundamental macro-nutrient (Marschner, 1995). Many studies based on  $^{87}\text{Sr}$  and  $^{86}\text{Sr}$  isotopes ( $^{87}\text{Sr}/^{86}\text{Sr}$  ratio) were determined to trace its source origins and transfer

processes. In fact, in contrast to lighter stable elements, Sr isotopic ratios are theoretically not affected by kinetic and equilibrium fractionations during low temperatures and biologic processes, due to their high atomic mass and small mass difference (Capo et al., 1998; Faure and Mensing 2005). Only a few data outlined very discrete Sr isotope fractionation in soil environments (Fietzke and Eisenhauer, 2006; Halicz et al., 2008; Shalev et al., 2013) and during uptake by plants (De Souza et al., 2010). These studies focused on stable  $^{88}\text{Sr}$  and  $^{86}\text{Sr}$  isotopes, which are not considered here.

Compilation of literature data focuses on two main sources for Sr supply, one derived from bedrock weathering and the second from exogenous atmospheric input (Graustein and Armstrong, 1983; Graustein, 1989; Miller et al., 1993; Stewart et al., 1998; Vitousek et al., 1999; Probst et al., 2000; Whipkey et al., 2000; Poszwa et al., 2004, 2009; Reynolds et al., 2012; Hartman and Richard, 2014; Clauer and Semhi, 2016). Recently, an additional potential source has been suggested in the context of forest ecosystems: ground aquifers whose solute elements are able to rise towards the roots system by capillary (Grigal and Ohman, 2005; Bedel et al., 2016).

The atmospheric source of Sr, that is to say Sr that is brought by precipitation or wind to a vegetable, is considered as major for some authors (Graustein and Armstrong, 1983; Graustein, 1989; Fichter et al., 1998; Chadwick et al., 1999; Probst et al., 2000; Poszwa et al., 2004, 2009; Maurer et al., 2012; Hartman and Richard, 2014). Atmospheric source material can be of natural continental or marine origin (precipitation, dust...) or of anthropogenic origin (sprayed fertilizer, pollutant particles, ...), and can be used by a plant either through its roots or its leaves (Clauer and Semhi, 2016). For instance,

rainwater transferred into soils can be a determining carrier, picked up by the roots system. Foliar absorption from atmospheric deposition can also lead to element mobilization and base cation accumulation in trees (van Der Heijden et al., 2013). Even, when reaching the soil, these deposits can constitute an important nutrient source for plants. Shallow rooting plants (annuals and perennials) that utilize water and nutrients from upper soil layer are thus more likely to be affected by atmospheric deposition of bioavailable strontium than deep rooting plants (Poszwa, et al., 2004, 2009; Hartman and Richards, 2014). It is thus assumed that atmospheric contribution gradually decreases with soil depth (Capo et al., 1998; Vitousek et al., 1999; Hartman and Richards, 2014). To be also mentioned is the fact that bioavailable Sr may depend on the possibility to be supplied by alterable minerals (Reynolds et al., 2012; Miller et al., 1993; Vitousek et al., 1999; Hartman and Richards, 2014; Song et al., 2015; Fichter et al., 2015). This latter contribution is probably more important in an old carbonate-rich bedrock. In sum, the contribution of each source seems to depend on varied parameters such as the plant species, the geological environment, and/or the hydrological context that can be calculated using a simple mixing equation (Stewart et al., 1998).

Two main reservoirs are generally considered for bioavailable Sr in the literature. They are referred as: (i) the free solution or mobile water of the soil, and (ii) the labile fraction of the soil (Capo et al., 1998; Stewart et al., 1998). The former free or mobile water of the soil is mainly represented by cations from solutes of the interstitial water, and from carbonates and other easily soluble minerals. Rainfall and irrigation waters potentially contribute to this fraction. On an operational point of view, the mobile fraction is basically



obtained by leaching soil samples with a weak acid or with purified water. The labile fraction of a soil is generally considered to consist of the 'exchangeable' cations that are removable from soil components. On an operational point of view, this 'exchangeable fraction' corresponds to cations that are leached from solid soils with buffered solutions such as ammonium acetate, ammonium chloride or ammonium nitrate (Tessier et al., 1979; Stewart et al., 1998; Takeda et al., 2006, 2008; International Organization for Standardization 19730, 2008). This method extracts cations from pore waters and those bounded to exchange sites on minerals and organic matter. The Sr content of this fraction is clearly linked to the nature of the geological bedrock and to the pedogenic processes that combine mainly the primary minerals alteration and the availability of the organic matter, but it is also controlled by rainfall (Chadwick et al., 1999). Many consider the reservoir consisting of the exchangeable cations to be the major source of base cations for the uptake by the vegetation (Blum, 2000; Gastberger et al., 2000; Takeda et al., 2006; Durante et al., 2013; van der Heijden et al., 2013; Bedel et al., 2016).

A compilation of literature data leads to the conclusion that Sr supplied by the exchangeable fraction, and therefore potentially mobile fraction, does clearly not result from a single function and that numerous parameters linked essentially to the plants and/or their environments could modify the behavior of a plant in its capacity to extract Sr preferentially from one or the other of the identified reservoirs. For instance, the plant variety as well as the geological and environmental contexts are determining parameters in the characterization of the bioavailable Sr. A combined contribution of these parameters can interfere in the bioavailable reservoir (Durante et al., 2013). Furthermore, few is known about the Sr bio-geological cycle in agricultural

environments, whereas its isotope signature is often considered as a reliable tracer of the geographic origin of agri-food products, by assuming that it is mainly derived from geological reservoirs of the cultivated area (Almeida and Vasconcelos, 2001; Barbaste et al., 2002; Fortunato et al., 2004; Swoboda et al., 2008; Techer et al., 2011; Voerkelius et al., 2010; Durante et al., 2013; Hartman and Richard, 2014; Vinciguerra et al., 2015; Petrini et al., 2015). However, little is still known about the contribution of the agricultural practice on the bioavailable reservoir, especially the impact of irrigation and fertilization. Hartman and Richards (2014) identified different Sr sources in plants with a clear dependence of some plant  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios with that of the atmospheric precipitations, which has also been suggested by Clauer and Semhi (2016). Constituents of fertilizers, such as Sr in the present case, can also be taken up by plants, even if their impact on the chemical composition of agri-food products is debatable (Srek et al., 2010; Techer et al., 2011; Gao et al. 2011).

## **2 The study sites**

### **2.1 Geographic setting and geological data**

The sites of this study are located in southern France, to the South-East of Nîmes (X: 43°40'59.99"; Y: 4°25' 59.99"). They correspond to two plots of olive tree separated by about 1 kilometre and grown on the same geological unit (Fig.1). The first corresponds to cultivated plots that belong to a regional olive oil mill called "The Costières Mill"

(<http://www.oliveraie-jeanjean.com/>). The second belongs to a regional laboratory, the Serfel station, dedicated to a nursing and production experimentation of stone fruits and vegetables (Fig. 1).

The two sites are located in the Mediterranean sedimentary basin, precisely in 'the Costières' area that represents a distinct geographical entity forming a plateau at an altitude of 30 to 80 m above sea level, bordering the Cretaceous limestones to the west (Fig. 1). The geological bedrock is characterized by an impermeable clayey sediment represented by grey-blue clay of Plaisancian age (3.6-2.6 My). This formation is topped by Rhone and Durance gravels of Pliocene-Quaternary age (Villafranchian, 0.9-3.6 My) consisting of heterogeneous materials (pebble of quartzite and quartz, limestones, magmatic rocks, cherts and flints, and metamorphic rocks in smaller proportions) (Desaunettes & Vigneron 1958; Barrière et al., 1973).

The soil of this area is a pebble soil called locally 'the gress'. It is quite heterogeneous in its mineralogical and granulometric composition, as confirmed by the ground of the studied Serfel zone (Fig. 2). Data on the amount and distribution of the <2 mm fraction reveal a metric zonation in the soils of the experimental plots. The fine fraction is dominant (more than 70%) in the central part, from the surface-until 75 to 100 cm depth. On the eastern and western parts of the plot, the fine fraction is concentrated in the 0-50 cm zone, even sometimes in the upper 0-25 cm zone. The 'gress' is classically known to be carbonate free, however  $\text{CaCO}_3$  has been detected in some parts of the experimental plot (Fig. 2).

## 2.2 Agricultural practices

### *At “The Costières Mill” site*

The olive oil produced by “The Costières Mill” has been recognized since 2011 by the label “Protected Designation of Origin” (PDO) that guarantees the geographical origin, the quality, and the know-how production and manufacturing of the product. The PDO label gives these oils a guaranteed market value. In this context, the Costières Mill is especially interested in the design of a tool able to discriminate its production from any other (Medini et al., 2015).

The *Olea europaea* species that includes the Cendre, Aglandau and Picholine varieties were planted in 1996 on the property of the mill. Today, the cultivated area extends over 30 hectares and is divided into four plots (D, E, F and G) for the purpose of the mill organization, with about 10,000 trees (Fig. 1). Since 2008, the holding has been converted to an organic production and obtained the “Organic Agriculture” label in 2011. Only specific treatment products are authorized to compile with this label; those used on the plots were of three types:

- a cuprous oxide is used as a fungicide sprayed on the trees during March;
- at about the same time, an organic fertilizer consisting essentially of dried blood and called the *Biovie* is deposited at the tree feet (about 350g/tree); and
- a white clay (kaolinite) is sprayed on the trees during summer (July) to avoid the olive fly called *Dacus Oleae* at an amount of 80 kg/ha.

Irrigation of “The Costières Mill” holding is based on a drop-by-drop supply using fluvial water drained from the Rhone River by the Bas-Rhone Languedoc channel. However, it was not possible to obtain the amounts of water flooded over the plots during the investigated period.

#### *At the Serfel site*

One of the research domains of the Serfel station concerns the cultivation of *Olea europaea* and more precisely the understanding of the irrigation impact on the olive production and the quality of the olive oil. The plots are located in the same geographic and geological area than “The Costières Mill” distanced by only about 1000 meters (Fig. 1). Two varieties of olive trees, Picholine and Aglandau, were planted in 2003 in dedicated plots over a surface of 11,340 m<sup>2</sup> (Fig. 2). Now, 315 specimens of about ten-years old trees are cultivated under known and controlled conditions with only specified irrigation modalities. The irrigation is activated according to soil humidity measurements monitored by two tensiometers buried at -35cm and -70cm depth for each modality. Irrigation is initiated automatically drop-by-drop, when humidity is at a minimal value of 60-70 cbars at -35cm in the soil. This supply is completed, as for “The Costières Mill”, by using fluvial water drained from Rhone River by the same Bas-Rhone Languedoc channel.

Two irrigation modalities are currently applied (Fig. 2):

- Modality 1 consisting in a supply of 25 mm of water at the threshold value of 65 cbars that corresponds to ‘high’ irrigation applied to the A and B plots.
- Modality 2 applied the same day than the previous one at the C and D plots, but considered as ‘moderate’ with a water supply reduced by half at 12.5 mm.

A supplementary control identified as group  $\phi$  with no watering other than the rain was also included.

Each group consisting of ten trees of the Picholine variety and ten trees of the Aglandau variety has been triplicated (Rep1, Rep2 and Rep3) (Fig. 2). To avoid border effects, only the 6 central trees of each given group were selected, including 3 Aglandau and 3 Picholine trees (Fig. 2). Three replicates of the same distribution were examined for the groups B (high irrigation modality), D (moderate irrigation modality), and  $\Phi$  (no anthropogenic irrigation).

Well under control, fertilization was identical for all trees especially the supplied amount of nitrogen. Two main plant-health products were used:

- (1) a mixture of Cu sulphate and Ca hydroxide (80%  $\text{Ca(OH)}_2$  + 20 %  $\text{CuSO}_4$ ) called the “Bordeaux mixture” sprayed on the trees before blooming (March) and after harvest (beginning of October), to prevent from specific disease of olive-tree called the ‘peacock eye’, and to prevent from specific bacterium and fungus;
- (2) as for “The Costières Mill”, white clay (kaolinite) was sprayed twice a year on the trees during summer (July – August) to avoid the olive fly (*Dacus Oleae*) at an amount of 50 kg/ha.

### **3 Sampling and Methods**

#### **3.1 Sampling**

##### *3.1.1 “The Costières Mill” plots*

Sampling of “The Costières Mill” site was completed in September 2012 at a restricted area of plot F (Fig. 1). Four specimens of Picholine and four specimens of Aglandau trees were selected not far from each other to avoid or, at least, reduce soil heterogeneities. Four branches of about 3 mm in diameter were cut on each tree at 1 m from soil. Leaves and olives were removed from each branch. For an extrapolation of the results at the 30-hectare scale of “The Costières Mill” site, 10 leaves were collected on each olive tree of the four plots (D, E, F, and G) that consist of about 2,000 to 3,500 trees per plot, and were mixed to build one leave-sample per plot. Olives collected during the harvest period were also sampled on the studied trees.

Soil samples of plot F were also collected using an auger, at a depth of 30 to 40 cm and distant by about 1 m from each of the 8 studied trees. As the site was operated at the time of sampling, it was not possible to collect the soil as a function of depth. The choice of the 30-40 cm depth was decided to avoid litter effects that might interfere in the recycling of strontium and other nutrients (Graustein and Armstrong, 1983). Also, as the root system of olive trees is known to be mostly a vertical and deep system (Kailis and Harris, 2007), the upper soil level was not expected to be the nutrient one and, consequently, not the strontium source. As stipulated by Kailis and Harris (2007), no roots were observed in the 0-30 cm zone during digging.

### 3.1.2 *The Serfel plots*

The three irrigation groups, B (high irrigation), D (moderate irrigation) and  $\phi$  (no irrigation) and their three repetitions (Rep1, Rep2, Rep3) were sampled as follows. In

each repetition, 3 specimens of Picholine and 3 specimens of Aglandau trees were selected (Fig. 2): branches and leaves were collected over two consecutive years, in November 2012 and September 2013, to evaluate the time effect. As for “The Costières Mill”, four branches were cut off each tree at about 1 m from soil. The leaves were removed from each branch (about 30 leaves per branch). Olives could not have been harvest as used for the Serfel own measurements.

To mimic “The Costières Mill” sampling, soil samples were initially collected at a depth of -30 to -40cm in each repetition of the different studied groups in November 2012. In September 2013, three soil profiles were made in one repetition of each group at -30/-40cm, -60/-70cm and -80/-90cm depths.

The products for the plant protection and the fertilizers were collected for chemical and Sr isotopic measurements at each studied site. They include the white clay (AB) used at the “The Costières Mill” and the Serfel sites, the Bordeaux-mixture (BB) used only at the Serfel, the cuprous oxide (Cu<sub>2</sub>O-Cos) and the biovie (Biov-Cos) used only at the “The Costières Mill”. To constrain atmospheric inputs, a rain gauge was installed on the Serfel site, and rainwater (RW) was collected at the time of material sampling. Water used for irrigation and derived for the two sites from Rhone River, was also collected (IW at two different time periods at the two sites).

### **3.2 Sample preparation and analytical procedure**



The tree organs (leaves, branches, olives) were carefully washed by ultrasonic treatment in distilled water, dried and mineralized in a microwave (Ethos one –Milestone at the University of Nîmes, France) according to the procedure described by Medini et al. (2015).

The soil samples were crushed gently and treated according to two procedures:

- (1) an aliquot of the powder was leached with supra-pure 1 M acetic acid considering a 1:100 soil/solution ratio in order to extract the mobile fraction of the powder;
- (2) a second aliquot was treated for extraction of the exchangeable fraction according to the procedure of Takeda et al. (2006) using a  $\text{NH}_4\text{NO}_3$  solution with a 1:10 soil/solution ratio.

Irrigation and rainwaters were filtered (0.45  $\mu\text{m}$ ) and acidified ( $\text{HNO}_3$  6N) after sampling. They were then evaporated and their residues dissolved for Sr isotope treatment and analysis.

The two products for the plant protection and the fertilizers were dissolved in mixed hot acids ( $\text{HF-HCl-HNO}_3$ ). The soluble fraction was collected, as potentially available for the plant. An amount of 300 mg of the white clay and 200-250 mg of the Cu-product and Biovie mixture were leached with supra-pure acetic acid. The leachates were then evaporated for Sr isotopic and chemical measurements.

The dry residue of the treated samples was dissolved in nitric acid for Sr separation and purification on the Eichrom Sr-resin, following the Pin et al.'s (2003) procedure. Strontium was loaded on a Ta-filament and the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic ratio measured by

solid-source thermal ionization mass spectrometry (TIMS ThermoFinnigan Triton TI) (at the University of Nîmes, France). For control of the analytical accuracy, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the NBS987 standard was determined periodically, giving an average of 0.710257 with a standard deviation of  $3\text{E-}6$ . The internal analytical uncertainty ( $2\sigma\text{D}$ ) was calculated for each measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio on the basis of 100 runs pro analysis. For groups of  $n$  samples, a median  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio and the standard deviation ( $2\text{SD}$ ) were calculated. Sr contents were determined for a few samples of the soils, the leaves and for the plant-protection products by inductively coupled plasma-mass spectrometry (ICP-MS at the Laboratoire d'Hydrologie et de Géo chimie of the University of Strasbourg) with an analytical precision of  $\pm 5\%$ , based on the procedure by Samuel et al. (1985).

## 4 Results

### 4.1 Branches, leaves and olives

Leaves and branches of 8 trees from F plot of "The Costières Mill" site were analyzed. Leave-samples consisting of 10 leaves per tree of each plot were also selected at the scale of the entire site (D, E, F and G plots) (Table 1 - Supporting Material). The overall database of the Sr isotopic composition of the branches and leaves was summarized statistically in box plots that outline the spread and average value of the trees from each compartment as a function of the location (Fig. 3a). The  $^{87}\text{Sr}/^{86}\text{Sr}$  values of the branches range from 0.708281 ( $\pm 8\text{E-}6$ ) to 0.70875 ( $\pm 10\text{E-}6$ ) with a median ratio of 0.7087 ( $\pm 1.6\text{E-}4$ ) (Fig. 3a). The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the leaves from the 8 trees yield the same range, from 0.708305 ( $\pm 1.6\text{E-}6$ ) to 0.70877 ( $\pm 13\text{E-}6$ ) with a median value of 0.7086 ( $\pm 1.6\text{E-}4$ )

(Fig. 3a). Values obtained for the tree leaves of the 4 plots are of the same order of magnitude with a median ratio of 0.7087 ( $\pm 1.2\text{E-}4$ ) (Fig. 3a). As a main observation, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of branches and leaves from trees of the “The Costières Mill” are identical with a median value of 0.7087 and a standard deviation of  $1.6\text{E-}4$ . Olives that could be sampled during the harvest period from 4 studied Aglandau trees (A4-1, A4-5, A4-10, A4-15) display also identical isotopic ratio ranging from 0.7083 ( $\pm 2\text{E-}5$ ) to 0.7086 ( $\pm 1\text{E-}5$ ) with a median value of 0.7086 ( $\pm 1.7\text{E-}4$ ) ( $n=4$ ). Alternatively, the Sr contents in the leaves are quite scattered ranging from 14.3 to  $41.4 \mu\text{g.g}^{-1}$ .

At the Serfel site, 27 samples of branches and 27 samples of leaves were analyzed for their Sr isotope composition combined with their Sr contents (Table 1 - Supporting Material). The Sr contents in the leaves range from 25 to  $44.5 \mu\text{g.g}^{-1}$ . The  $^{87}\text{Sr}/^{86}\text{Sr}$  values of the branches range from 0.708927 ( $\pm 6\text{E-}6$ ) to 0.709319 ( $\pm 7\text{E-}6$ ) with a median ratio of 0.7091 ( $\pm 1.0\text{E-}4$ ) ( $n=27$ ) (Fig. 3a). The Sr isotopic ratios in the leaves define the same narrow range, from 0.709047 ( $\pm 6\text{E-}6$ ) and 0.70945 ( $\pm 12\text{E-}6$ ) with a median value of 0.7092 ( $\pm 1.1\text{E-}4$ ) ( $n=27$ ) (Fig. 3a). Branches and leaves from the Serfel plots display identical  $^{87}\text{Sr}/^{86}\text{Sr}$  values with a median value of 0.7092 and a standard deviation of  $1.1\text{E-}4$ .

These results outline: (1) an identical  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio in the studied organs of the trees for a given agricultural site, (2) a significant difference between the Sr values of the Serfel and “The Costières Mill” trees. Also to be mentioned are the identical Sr isotopic compositions of the Picholine and Aglandau tree varieties of the same plots, which

suggest in turn that the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios are not tree-type dependent (Table 1 - Supporting Material).

If combining branches and leaves of a given site because of their isotopic identity, very tiny variations can be observed at the Serfel site as a plausible function of the irrigation modality. Indeed, slightly higher  $^{87}\text{Sr}/^{86}\text{Sr}$  values have been measured in samples removed from trees cultivated under moderate irrigation conditions (D modality) (Fig. 3b). The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the branches and leaves taken from trees that have been either highly irrigated (B modality) or not irrigated ( $\phi$  modality) are slightly lower and within the same order of values (Fig. 3b). However, on a statistical point of view, all data are identical with median values of 0.7092 ( $\pm 1.1\text{E-}4$ ), 0.7092 ( $\pm 1.0\text{E-}4$ ), and 0.7091 ( $\pm 1.0\text{E-}4$ ), respectively for the trees subjected to not, moderate and high-irrigated conditions.

### 3.2 Soils

The irrigated soils collected at “The Costières Mill” site yield low Sr contents in both the mobile or exchangeable fraction, ranging from 4.52 to 8.28  $\mu\text{g.g}^{-1}$ . The two fractions yield the same  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios with a median value of 0.7085 and a standard deviation of 1.1 and 5.0E-5 for the mobile and the exchangeable fraction, respectively (Table 2 - Supporting Material; Fig. 4a). This value is close to that measured in the organs of the trees (branches, leaves, olives) of this cultivated site ( $0.7087 \pm 1.6\text{E-}4$ ) (Fig. 4a).

At the Serfel site, the mobile and exchangeable fractions of the soils yield distinct Sr elemental contents and isotopic values as a function of the irrigation modality. Both fractions of the irrigated soils (moderate and high irrigation modalities) yield low Sr contents for the ranging from 4.94 to 16.3  $\mu\text{g.g}^{-1}$  with a median value of 8.29  $\mu\text{g.g}^{-1}$  (Table 2 - Supporting Material). Conversely, the mobile fraction of the not-irrigated soils is characterized by higher Sr contents, ranging from 17.2  $\mu\text{g.g}^{-1}$  to 41.7  $\mu\text{g.g}^{-1}$  with a median value of 30.2  $\mu\text{g.g}^{-1}$ . On each studied plot, higher Sr contents are measured at the -30-40 cm depth of the soil profiles. On the Sr isotopic point of view, the mobile and exchangeable fractions of the soils are significantly different for the non-irrigated plot with  $^{87}\text{Sr}/^{86}\text{Sr}$  median values of 0.70854 (3.2E-5) and 0.70897 (4.4E-5), respectively (Fig. 4a). These values are clearly lower than the median Sr composition measured in the organs of trees cultivated in these plots ( $0.7092 \pm 1.1\text{E-}4$ ). Under moderate irrigation, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios measured in the mobile and exchangeable fraction of the soils range from 0.708811 to 0.709296 and from 0.70902 to 0.70928 with identical median values of 0.7090 (1.7E-4) and 0.7092 (1.1E-4), respectively (Fig. 4a). These ratios are close to those measured in the tree organs of the trees grown in the moderately irrigated plots ( $0.7092 \pm 1.0\text{E-}4$ ). Similarly, soils from the high-irrigated plots display  $^{87}\text{Sr}/^{86}\text{Sr}$  values for their mobile and exchangeable fractions that recall those measured in branches and leaves of trees (Fig. 4a). The isotopic ratios of the mobile fraction range from 0.70863 to 0.70899 with a median value of 0.7088 (1.7E-4), whereas the exchangeable fraction yields a median ratio at 0.70904 (7E-5) that is less scattered, ranging from 0.70892 to 0.70904.

If considering the mobile soil fractions, heterogeneous and distinct  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios are observed as a function of location ("The Costières Mill" or the Serfel), of irrigation modality, and for the same modality as a function of depth in the soil (Fig 4b). At the upper levels (-30/-40cm), the  $^{87}\text{Sr}/^{86}\text{Sr}$  values of the mobile phase of the soils from not-irrigated plots of the Serfel site are the lowest, ranging from 0.708509 ( $\pm 6\text{E-6}$ ) to 0.708605 ( $\pm 10\text{E-6}$ ) (Fig. 4b). These values are close to those measured in "The Costières Mill" soils (Fig. 4b), while clearly lower than those measured in the highly irrigated soils where they range from 0.708631 ( $\pm 10\text{E-6}$ ) to 0.708997 ( $\pm 4\text{E-6}$ ), and even more in the moderate irrigated ones, from 0.708811 ( $\pm 10\text{E-6}$ ) to 0.709045 ( $\pm 16\text{E-6}$ ) (Fig. 4b). When considering the distribution of the  $^{87}\text{Sr}/^{86}\text{Sr}$  values relative to depth, varied outlines can be distinguished: whereas values are quite homogeneous and identical from -30 to -80 cm in the not-irrigated plots (median value of  $0.70853 \pm 3.2\text{E-5}$ ), they clearly tend towards higher ratios when irrigation is moderate and high. A high heterogeneity in the values is also visible for samples collected at a same depth for the high irrigation modality at "The Costières Mill" site.

Considering the exchangeable fraction of the same soils, distinct values are again obtained in the two sites: the exchangeable soil fractions of "The Costières Mill" site is characterized by low isotopic compositions compared to those of the Serfel site with a median  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of 0.70852 ( $5\text{E-5}$ ) (Fig. 4c). The exchangeable fraction of the soils from Serfel site are less scattered compared to those of the mobile fraction, ranging from 0.70888 ( $3\text{E-5}$ ) to 0.70904 ( $1.3\text{E-5}$ ) in the upper collected soil levels, increasing with depth to a value of 0.70928 ( $2.6\text{E-5}$ ).

A comparison of the Sr isotopic composition from exchangeable fraction with that from mobile one provides similar values for the soils of “The Costières Mill” and for those of the Serfel site that were moderately and highly irrigated (Fig. 4c). Conversely, specific trends are observed in the two fractions of the not-irrigated soils: the exchangeable fraction appears to be more radiogenic than the mobile one. As a conclusion, the mobile and the exchangeable fraction of a soil yield quite the same  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in irrigated conditions, whereas clearly distinct ratios when the soils are not irrigated.

#### 4.3 Waters, plant protection products and fertilizers

Water used for irrigation at the Serfel and “The Costières Mill” sites yields a mean  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of 0.708539 ( $\pm 9\text{E}-6$ ). Rainwaters collected in the Serfel rain gauge are slightly more radiogenic with an  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of 0.70877 ( $\pm 1\text{E}-5$ ).

The Sr isotopic and chemical compositions of the plant-protection products were determined on the whole material (total dissolution of the products labeled WR), and on the leachable component (labeled SP) (Table 3 - Supporting Material). The white clay used at both sites, yields high radiogenic  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios whatever the studied fractions (WR = 0.758760 ( $\pm 8\text{E}-6$ ) and SP = 0.74325 ( $\pm 4\text{E}-5$ )). The cuprous oxide and the Biovie used on the olive trees at “The Costières Mill” are also characterized by high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios (WR=0.713091 ( $\pm 5\text{E}-6$ ) SP from 0.71098 ( $\pm 2\text{E}-5$ ) to 0.70950 ( $\pm 2\text{E}-5$ )). The Bordeaux mixture used at the Serfel site appears to be the less radiogenic anthropogenic product with  $^{87}\text{Sr}/^{86}\text{Sr}$  values of 0.708095 ( $\pm 7\text{E}-6$ ) and 0.70801 ( $\pm 2\text{E}-5$ ).

for the whole product and the leachable fraction, respectively. The Bordeaux mixture and the white clay contain quite high Sr contents of about 107 and 68  $\mu\text{g.g}^{-1}$ , whereas the Cu-oxide used during the cultivation is very poor in this element (2  $\mu\text{g.g}^{-1}$ ).

## 5. Discussion

The Sr geochemical characteristics of olive trees were determined in two domains identical in terms of geographic and soil and rock contexts, but distinct in the applied cultivation modalities, the pedologic, geologic and climatic parameters being identical in the two sites. If considering that the bio-available Sr has a natural origin, that is to say is extracted either from soil minerals or from rainwaters (Stewart et al., 1998), the same Sr isotopic composition is expectable in the tree organs of the two sites. Quite varied  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were measured: at the Serfel site, olive-tree branches and leaves yield Sr isotopic composition clearly higher ( $0.7092 \pm 1.1\text{E-}4$  (n=54)) than those recorded in the same organs of “The Costières Mill” trees ( $0.7087 \pm 1.6\text{E-}4$  (n=20)) (Fig. 3). As the geological and climatic environments are the same for the two sites, and as the same olive varieties were studied in the two cases, the potential contribution of agricultural parameters must be considered to explain the difference in the Sr composition. The cultivation modalities had, therefore, to impact significantly the source of the bioavailable Sr. In order to better constrain this hypothesis, the contribution of Sr from soils, natural waters and humans-added components (irrigation waters, fertilizers and protection products) to the olives trees is discussed further on the basis of the Sr isotopic and elemental patterns depending on the applied cultivation modalities.



### 5.1 The Sr isotopic composition of olive trees

Considering the statistical data, branches and leaves of trees yield homogeneous and identical Sr isotopic compositions for these organs at a given site (Fig. 3a) and no distinction can be made between the two varieties of *Olea europaea* (Picholine and Aglandau). As already stated above, this identity supports the same biological behavior of Sr taken up by the Picholine and Aglandau tree varieties, excluding a potential control by the plant species. To pursue this aspect, a comparison can be made with Sr isotopic measurements obtained on olive oil produced by “The Costières Mill” (Medini et al., 2015). The authors defined a Sr isotope method for olive oil characterization and analyzed various types. They published an  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of 0.7088 ( $\pm 2\text{E-}4$ ) for the olive oil produced by “The Costières Mill” in 2012. This value is identical to the Sr isotopic ratios measured in the branches, leaves and olives of the trees grown at this site.

Despite the statistical Sr isotope homogeneity at each site, a specific care may be paid to branches and leaves Sr isotope data obtained from trees of the Serfel not-irrigated plots, and to a lesser extent from those of the highly irrigated ones (Table 1 - Supporting Material; Fig. 5). Even if the two types of samples yield statistically the same Sr isotope composition, slightly higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were determined in leaves compared to branches. This slightly higher Sr isotopic composition implies addition of a Sr with a higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio to the leaves that can be explained by deposition of an external, atmospheric compound with a higher ratio. Foliar transfer of elements has already been

described (Scotti and Carini, 2000; van der Heijden et al., 2013; Schreck et al., 2014; Bedel et al., 2016 and references therein). For instance, Ca absorption by the foliage in broadleaf forests was reported and even calculated (van der Heijden et al., 2013). The same mechanism can be suggested for Sr being an analogous element of Ca. In this case, candidates for atmospheric deposition are either of natural origin such as aerosols carried by rainwater, or of an anthropogenic origin such as the sprayed agricultural products. Plain rainwater collected during the study period yields a low  $^{87}\text{Sr}/^{86}\text{Sr}$  value of 0.70877 ( $\pm 1\text{E-}5$ ), lower than that of the leaves and cannot, thus, be considered as a good candidate for this external Sr. Rainwaters are however known to have  $^{87}\text{Sr}/^{86}\text{Sr}$  compositions that are susceptible to vary significantly in time and space. For instance, values ranging from 0.709198 and 0.713143 were measured in rainwater samples collected in the Massif Central (France) over a period of one year (Negrel and Roy, 1998). Despite these data, an impact of  $^{87}\text{Sr}$ -rich rainwater is not expected here as not identified at "The Costières Mill" site. Apparently, natural deposits do not explain the slight Sr isotopic shift between leaves and branches observed at the Serfel site. Alternatively, other components that may potentially have been deposited on the leaves are the sprayed plant protection products (e.g., the white clay and Bordeaux mixture at Serfel). As for rainwater, the Bordeaux mixture appears to yield lower  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios than the tree organs and cannot have induced the observed increase in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio. The white clay protection product represents in the Serfel environment context the sole potential reservoir enriched in  $^{87}\text{Sr}$ . The bulk component and its soluble fraction display significantly high  $^{87}\text{Sr}/^{86}\text{Sr}$  values (Table 3 - Supporting Material), and even a limited contribution of this product can induce a noticeable increase of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio. Sprayed on the trees, this product could have adhered to the leaves better than to the

branches due to the greater contact surface, and may even have penetrated into the stomata of the leaves. This is observed at the Serfel site, whereas it was not identified in the samples from “The Costières Mill” site, where the same protection product is used. The insignificant  $^{87}\text{Sr}$  increase for the Sr taken up by tree leaves at “The Costières Mill” site may then relate to a lower amount of white clay used in the ‘biological’ modality. During a cultivation year, the white clay was sprayed once on “The Costières Mill” trees (80Kg/ha), whereas added twice at those of the Serfel site ( $2 * 50\text{kg/ha}$ ). Finally, the fact that moderate irrigated trees at Serfel do not display the  $^{87}\text{Sr}$  enrichment in leaves may be due to the spreading procedure. In turn, the trees located in the central parts of the plots may have received less production products than those around.

Homogeneous on a given site, the median  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios values of the tree organs are, however, clearly distinct in the “The Costières Mill” and Serfel sites. This implies different Sr sources as a function of the site and of the cultivation modality.

## **5.2 Origin of Sr in tree organs and implication for a geographical discrimination of olives and olive oil**

### *“The Costières Mill” site*

The relationship between the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the organs from olive trees (leaves and branches) and those of the soils and waters reservoirs at the “The Costières Mill” site can be discussed as a function of a vertical projection (Fig. 5). Considering the biologic agriculture practices applied at this site, olives, leaves and branches yield  $^{87}\text{Sr}/^{86}\text{Sr}$  values that are of the same order than those of either the mobile or exchangeable soil

fractions, being also identical to those of the irrigation waters and, to a lesser extent, to that of the rainwaters. On the basis of the cultivation practice at “The Costières Mill” site, a clear, simple and straight relation is thus obtained between the Sr isotopic composition of the tree organs and that of their growing environment. The soil appears to be the major source of base cations for the trees, either through its mobile or exchangeable fractions. It seems that the used anthropogenic products have no impact on the Sr isotopic composition of the trees. This relationship between the trees and their natural environment is of special interest when using the Sr isotope composition as a tracer of the geographic origin of olives and olive oil. For instance, Medini et al., (2015) observed by means of geological map studies and oils analyses a fair correlation between the Sr isotopic composition of an olive oil and the geologic bedrock-of the producing orchards. In fact, we demonstrate this relationship in the herein study. In turn, one can assume the origin of olive trees grown on a specific soil by analyzing the Sr isotope composition of the olives and of the derived olive oil, and comparing the results with the Sr isotope composition of the soil in the case of a biologic farming with drop-by-drop irrigation. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of these agri-foods can thus be used to discriminate the production area of tree nurses located on distinct bedrocks.

#### *The Serfel site*

Despite the similar soil and climatic conditions, trees from Serfel site display higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios relative to those determined on “The Costières Mill” trees. Their Sr isotopic compositions are, however, homogeneous whatever the irrigation modality, which agrees with the fact that irrigation is not a predominant parameter in the definition

of the bio-available Sr source. Indeed, irrigation and rain-waters yield significantly lower  $^{87}\text{Sr}/^{86}\text{Sr}$  values than tree leaves and branches (Fig. 5) and, therefore, cannot be a major source for the bio-available Sr. Furthermore, no direct relation can be observed between the Sr isotope compositions of tree organs and mobile soil fractions. A better relation can be determined between the Sr of the tree samples and that of the exchangeable soil fractions, even if some tree organs can contain Sr with higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios than this exchangeable fluid phase. As a preliminary statement, cations available for the olive trees are of various origins and do not only reflect a natural origin in “conventional agricultural practices”, with or without irrigation. It seems that anthropogenic products have a significant impact on the elemental tree uptake in this agricultural context. The Sr isotopic composition of olive trees cannot be directly derived from natural environment, and can reflect a potential imprint supply of man-made anthropogenic products. Special care in the use of the Sr isotopic tool is, therefore, needed in discriminating studies.

### 5.3 Sr isotopic composition of soils

As for tree organs, distinct  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were measured in the mobile and exchangeable fractions of the two studied sites (Fig. 5), despite the proximate location and the similar geographic and geologic areas that were selected on purpose to avoid as much as possible heterogeneities due to variations of the geological and pedologic basement. Despite this choice and the homogeneous nature of the soils, slight mineralogical and structural differences were observed at the scale of the plots. At the Serfel site, even if being randomly distributed in space, the studied plots were

characterized by specific carbonate and fine fraction contents (Fig. 2). However, no specific correlation between the Sr isotopic composition of the mobile or the exchangeable fractions and the mineralogical and textural parameters could be detected. For instance, the three replicates of the not-irrigated plots are characterized by three different patterns in term of fine fraction distribution and by homogeneous  $^{87}\text{Sr}/^{86}\text{Sr}$  compositions of the mobile and exchangeable fractions. Similarly, the B-Rep1 and D-Rep1 plots that are characterized by different carbonates contents and similar granulometric texture are identified by close  $^{87}\text{Sr}/^{86}\text{Sr}$  compositions. In summary, the local soil heterogeneities seem not to control the Sr isotopic composition of their varied fractions. Therefore, another external parameter must be identified for the heterogeneities in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the soils at the Serfel site and, consequently, for the difference with those of “The Costières Mill”.

Variations in the mobile fraction of soils from various studied plots were observed in both the Sr isotopic ratios and elementary contents (Fig. 6). When the soils are not irrigated ( $\Phi$  plots of Serfel), their mobile fraction is characterized by high Sr contents (average value of  $30 \mu\text{g}\cdot\text{g}^{-1}$ ) and low  $^{87}\text{Sr}/^{86}\text{Sr}$  ( $0.70854 \pm 3\cdot\text{E}\cdot 5$ ). With irrigation (B and D plots of Serfel and “The Costières Mill”), this fraction appears to be Sr depleted with higher  $^{87}\text{Sr}/^{86}\text{Sr}$  compositions, which does not favor any clear relationship with the irrigation degree. The increase in the Sr isotopic values is especially visible at the Serfel site, even if it can also be observed in some samples at “The Costières Mill” (Fig. 6). The decrease in the Sr contents of the mobile fraction from these soils can be explained by: (1) a dissolution process of Sr-rich soluble minerals during irrigation and by irrigation

waters, and/or (2) an important root activity with metabolic processes of ions uptake by root cells in irrigated conditions (Marschner, 1995) (Fig. 6). These chemical or biochemical processes can induce the increase of the remaining phase by a balance effect. However, as mineralogical measurement are lacking, it is quite difficult to argue further. On the other hand, the increase of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from mobile fraction of irrigated soils calls for a supply of Sr with a higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio or an isotopic fractionation during the chemical or biological process that is clearly not conceivable as discussed above. In the hypothesis of a rich- $^{87}\text{Sr}$  supply, its impact appears stronger at the Serfel site (Fig. 6). Considering that Sr of irrigation water yields a low  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio, the observed increase in the  $^{87}\text{Sr}/^{86}\text{Sr}$  of the irrigated plots cannot be due to a supply of this water. In addition, such a supply was not identified at “The Costières Mill” site, which plots were also irrigated even if the irrigation degree was not specified.

To explain the nature and origin of this potential supply of Sr with an increased  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio, it is needed to focus on the main difference in the cultural modalities. This difference deals mainly with the use of fertilizers and protection products: the Bordeaux mixture was used at the Serfel site, whereas Cu-oxide and Biovie were applied at “The Costières Mill” site, white clay being used at both sites. These products sprayed on the trees or deposited at their feet are characterized by distinct Sr elementary and isotopic compositions (Table 3 - Supporting Material). As a consequence, their potential incorporation in the soils could induce a change of the intrinsic Sr pattern of the soil reservoir by a mixing process. Mixing between the fractions of the soils and the plant-health products can be illustrated in an  $^{87}\text{Sr}/^{86}\text{Sr} = f(\text{Sr})$  diagram where data from soils and anthropogenic products were reported (Fig. 6). In irrigated conditions, the Sr data of

the mobile fractions from the soils fit on a trend towards a reservoir characterized by a high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio that corresponds to the soluble fraction of the white clay product. This can be explained by the use of this product at the two sites, spread once or twice a year. During spreading, particles probably reach the soil and are dissolved, leading to a soluble phase that could have been transported by pore waters of the soil. As the leachate of the white clay has a low Sr content and a high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio, its addition to the soil does not induce significant variations of the Sr content from mobile fraction, but a clear increase of its isotopic value. The impact of this process is, thus, implemented by irrigation. Such an imprint of the white clay is clearly observed in the moderately and highly irrigated soils of the Serfel site. Alternatively, it is less pronounced in “The Costières Mill” soils despite irrigation (Fig. 6). This can be explained again by the cultivation practices, with more white clay spread on the trees at the Serfel site (2 times 50kg/ha/year) compared to “The Costières Mill” trees (80kg/ha/year). In addition, as already mentioned, penetration of the white clay soluble particles into the soil is favored by running rain- and irrigation water. At “The Costières Mill” site, a drop-by-drop irrigation system exists, but no information about the amount of used water was made available. On the basis of the low deviation of the Sr isotopic composition of these soils, a lower irrigation degree than that observed at the Serfel site is probable.

The exchangeable fraction of the Serfel soils seems also to have been impacted by the same process of white clay leached into the soluble fraction, whereas the Sr of that of “The Costières Mill” soils remains of the same value than those of the mobile fraction. A similar trend is observed in the diagram between the Sr contents and isotope compositions of the soil samples and the plant-protection products (Fig. 6). However,



the reported data do not exactly plot on the mixing trend defined with the leached phase of the white clay; they are slightly shifted to the right of the trend. It looks like another component enriched in Sr with a higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio contributes to the composition of these fractions of the soil from Serfel. Among the used anthropogenic products, bulk particles of white clay could be an appropriate candidate (Biovie being not used in the Serfel agricultural modalities) (Fig. 6). As these particles contain cations bounded to exchange sites, their addition to the soil suggests a disturbance of the intrinsic properties of the exchangeable fraction. The exchangeable phase of the Serfel cultivated soils appears thus to be imprinted by anthropogenic sources represented by the white clay. This is not observed at “The Costières Mill” due to the lower amount of used white clay, even if a slight Sr enrichment can be detected, probably involving the same mixing process. On the Sr elementary basis, the exchangeable fraction of the soils does not appear to have been significantly disturbed by the previously discussed dissolution of Sr-rich phases. All studied soil samples irrigated or not of both “The Costières Mill” and the Serfel site, display quite the same Sr contents (Fig. 6).

The nature of the protection products added to the cultivated plots can be responsible for significant changes in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the soil extractable fractions, either mobile or exchangeable. For instance, the use of a product that contains Sr with a high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio, such as the white clay, can lead to an increase of this ratio in the two phases. Irrigation is a cultural practice that leads to depletion of the Sr contents of the mobile fraction, whereas it has no significant effect on the exchangeable fraction.

## 6. Conclusion

The role of cultivation practices on bio-available  $^{87}\text{Sr}/^{86}\text{Sr}$  of olive trees is discussed on the basis of a Sr elementary and isotope study conducted on tree organs of two sites located about 1 km apart on similar soils, but with distinct cultivation modalities. Aglandau and Picholine olive trees contain Sr with identical  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in varied organs (branches, leaves, olives) at the scale of the studied areas. The Sr isotopic composition of the trees is clearly related to the soil mineral compositions in case of a biologic agriculture. However, it can also be significantly disturbed in the case of 'conventional cultivation' modalities. Irrigation is a widely used agricultural practice that can lead to the dissolution of Sr soluble-mineral and, as a consequence, to lower Sr contents of the mobile fractions from soils. It is also responsible for the transfer of particles and soluble phases from plant-protection products into the soils. This process can lead to the disturbance of the intrinsic Sr isotopic properties of both the exchangeable and mobile fractions. For instance, white clay used here to protect the trees from 'olive fly' disease induces an increase of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the mobile and exchangeable fractions of the soils. This imprint is observed only under 'conventional agricultural modality' that implies high spreading of white clays on the trees, and it is not detectable in a 'biological agriculture' modality.

Finally, the  $^{87}\text{Sr}/^{86}\text{Sr}$  measured in organs of olive trees and in the consumable products is dependent on their growing environment and on the applied cultivation practices. Thus, the mechanisms involved in the Sr uptake by olive trees can be used as a reliable tool to trace the origin of the olive and olive oil production, but only in case of a 'biologic'

agricultural practice that does not need an intensive use of additive anthropogenic products during the growth process of the plants.

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## References

- Almeida CM., Vasconcelos MTSD. (2001) ICP-MS determination of strontium isotope ratio in wine in order to be used as a fingerprint of its regional origin. *Journal of Analytical Atomic Spectrometry* **16**, 607–11.
- Barbaste M., Robinson K., Guilfoyle S., Medina B., & Lobinski R. (2002) Precise determination of the strontium isotope ratios in wine by inductively coupled plasma sector field multicollector mass spectrometry (ICP-SF-MC-MS). *Journal of Analytical Atomic Spectrometry* **17**, 135–137
- Barrière J., Bousquet J.C., Toni C. (1973) Données nouvelles sur la néotectonique des Costières du Gard. *C.R. Acad. Sci., Paris*, **277**, 285-288.
- Bedel L., Poszwa A., van der Heijden G., Legout A., Aquilina L. and Ranger J. (2016) Unexpected calcium sources in deep soil layers in low-fertility forest soils identified by strontium isotopes (Lorraine plateau, eastern France). *Geoderma* **264**, 103–116.
- Blum, J. D., Taliaferro, E. H., Weisse, M. T. and Holmes R. T. (2000) Changes in Sr/Ca, Ba/Ca and Sr-87/Sr-86 ratios between trophic levels in two forest ecosystems in the northeastern USA. *Biogeochemistry* **49**, 87–101.
- Capo, R.C., Stewart, B.W., Chadwick O. A. (1998) Strontium isotopes as tracers of ecosystem processes: theory and methods. *Geoderma* **82**, 197–225.
- Chadwick O. A., Derry L. A., Vitousek P. M., Huebert B. J. and Hedin L. O. (1999) Changing sources of nutrients during four million years of ecosystem development. *Nature* **397**, 491–497.
- Clauer N. and Semhi K. (2016) An evaluation of soil–plant–water interactions based on the  $^{87}\text{Sr}/^{86}\text{Sr}$ ,  $^{137}\text{Cs}/^{137}\text{Ba}$ ,  $^{137}\text{Cs}/^{137}\text{Sr}$ ,  $^{40}\text{K}/^{39}\text{K}$  and  $^{40}\text{K}/^{39}\text{Ca}$  ratios of the respective components. *Environ. Earth Sci.* **75**, 690.
- De Souza G. F., Reynolds B. C., Kiczka M. and Bourdon B. (2010) Evidence for mass-dependent isotopic fractionation of strontium in a glaciated granitic watershed. *Geochim. Cosmochim. Acta* **74**, 2596–2614.

- Desaunettes J.R., Vigneron J. (1958) Etude pédologique au 1/50.000 ème de la Costière du Gard et de la Vistrenque, *Annales Agronomiques* **t. VI**, 749-799.
- Durante C., Baschieri C., Bertacchini L., Cocchi M., Sighinolfi S., Silvestri M. and Marchetti A. (2013) Geographical traceability based on  $^{87}\text{Sr}/^{86}\text{Sr}$  indicator: A first approach for PDO Lambrusco wines from Modena. *Food Chem.* **141**, 2779–2787.
- Fairweather-Tait, S. and Hurrell R. F. (1996) Bioavailability of mineral and trace elements. *Nutr. Res. Rev.*, 295–324.
- Faure, G., Mensing, T.M. (2005) *Isotopes: principles and applications*. 3rd ed. New Jersey: John Wiley and Sons, Inc.
- Fichter J., Turpault M. P., Dambrine E. and Jacques Ranger (1998) Localization of base cations in particle size fractions of acid forest soils (Vosges Mountains, N-E France). *Geoderma* **82**, 295–314.
- Fietzke J. and Eisenhauer A. (2006) Determination of temperature-dependent stable strontium isotope ( $^{88}\text{Sr}/^{86}\text{Sr}$ ) fractionation via bracketing standard MC-ICP-MS. *Geochemistry, Geophys. Geosystems* **7**.
- Fortunato, G., Mucic, K., Wunderli, S., Pillonel, L., Bosset, J.O., Gremaud, G. (2004) Application of strontium isotope abundance ratios measured by MC-ICP-MS for food authentication. *J Anal At Spectrom.* **19**, 227–34
- Fuhrmann M. and Lanzirotti A. (2005)  $^{241}\text{Am}$ ,  $^{137}\text{Cs}$ , Sr and Pb uptake by tobacco as influenced by application of Fe chelators to soil. *J. Environ. Radioact.* **82**, 33–50.
- Gao X., Mohr R. M., McLaren D. L. and Grant C. A. (2011) Grain cadmium and zinc concentrations in wheat as affected by genotypic variation and potassium chloride fertilization. *F. Crop. Res.* **122**, 95–103.
- Gastberger M., Steinhäusler F., Gerzabek M. H., Lettner H. and Hubmer A. (2000) Soil-to-plant transfer of fallout caesium and strontium in Austrian lowland and Alpine pastures. *J. Environ. Radioact.* **49**, 217–233.

- Graustein, W.C. (1989)  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios measure the sources and flow of strontium in terrestrial ecosystems. In: Rundel PW, Ehleringer JR, Nagy KA, editors. *Stable isotopes in ecological research*. New-York: Springer-Verlag, 491–512.
- Graustein, W.C., Armstrong, R.L. (1983) The use of strontium-87/strontium-86 ratios to measure atmospheric transport into forested watersheds. *Science* **219**, 89–92
- Grigal, D.F., Ohmann, P.R. (2005) Calcium and forest systems: diffusion from deep sources. *Soil Sci.* **170**, 129–13.
- Halicz L., Segal I., Fruchter N., Stein M. and Lazar B. (2008) Strontium stable isotopes fractionate in the soil environments? *Earth Planet. Sci. Lett.* **272**, 406–411.
- Hartman G. and Richards M. (2014) Mapping and defining sources of variability in bioavailable strontium isotope ratios in the Eastern Mediterranean. *Geochim. Cosmochim. Acta* **126**, 250–264.
- Hinsinger, P. (1998) How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. *Adv. Agron.* **64**, 225-265.
- Hinsinger, P. (2001) Bioavailability of trace elements as related to root-induced chemical changes in the rhizosphere. In: Gobran, G.R., Wenzel, W.W., Lombi, E. (Eds.), *Trace Elements in the Rhizosphere*. CRC Press, Boca Raton, pp. 25-41.
- International Organization for Standardization 19730 (2008) *Soil Quality- extraction of Travel Elements from Soil Using Ammonium Nitrate Solution*, ISO, Geneva.
- Janin M., Medini S. and Técher I. (2014) Methods for PDO olive oils traceability: state of art and discussion about the possible contribution of strontium isotopic tool. *Eur. Food Res. Technol.* **239**, 745–754.
- Kailis S. and Harris D.J. (2007) *Producing Table Olives*. Landlinks Press, 2007 - 328 pages.
- Negrel P. and Roy S. (1998) Chemistry of rainwater in the Massif Central ( France ): a strontium isotope and major element study. *Appl. Geochemistry* **13**, 941–952.

- Marschner H. (1995) Mineral Nutrition of Higher Plants. Academic Press, London.
- Maurer A. F., Galer S. J. G., Knipper C., Beierlein L., Nunn E. V., Peters D., Tütken T., Alt K. W. and Schöne B. R. (2012) Bioavailable  $^{87}\text{Sr}/^{86}\text{Sr}$  in different environmental samples - Effects of anthropogenic contamination and implications for isoscapes in past migration studies. *Sci. Total Environ.* **433**, 216–229.
- Medini S., Janin M., Verdoux P. and Techer I. (2015) Methodological development for  $^{87}\text{Sr}/^{86}\text{Sr}$  measurement in olive oil and preliminary discussion of its use for geographical traceability of PDO Nîmes (France). *Food Chem.* **171**, 78–83.
- Miller, E.K., Blum, J.D., Friedland, A.J. (1993) Determination of soil-exchangeable cation loss and weathering rates using Sr isotopes. *Nature*, **362**, 438–441.
- Petrini R., Sansone L., Slejko F. F., Buccianti A., Marcuzzo P. and Tomasi D. (2015) The  $^{87}\text{Sr}/^{86}\text{Sr}$  strontium isotopic systematics applied to Glera vineyards: A tracer for the geographical origin of the Prosecco. *Food Chem.* **170**, 138–144.
- Pin C., Joannon S., Bosq C., Le Fèvre B. and Gauthier P. (2003) Precise determination of Rb, Sr, Ba, and Pb in geological materials by isotope dilution and ICP-quadrupole mass spectrometry following selective separation of the analytes. *J. Anal. At. Spectrom* **18**, 135–141.
- Poszwa, A., Ferry, B., Dambrine, E., Pollier, B., Wickman, T., Loubet, M., et al. (2004). Variations of bioavailable Sr concentration and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio in boreal forest ecosystems. Role of bio-cycling, mineral weathering and depth of root uptake. *Biogeochemistry* **67**, 1-20.
- Poszwa A., Ferry B., Pollier B., Grimaldi C., Charles-Dominique P., Loubet M. and Dambrine E. (2009) Variations of plant and soil  $^{87}\text{Sr}/^{86}\text{Sr}$  along the slope of a tropical inselberg. *Ann. For. Sci.* **66**, 512.
- Probst A., El Gh'mari A., Aubert D., Fritz B. and McNutt R. (2000) Strontium as a tracer of weathering processes in a silicate catchment polluted by acid atmospheric inputs, Strengbach, France. *Chem. Geol.* **170**, 203–219.

- Reynolds A. C., Quade J. and Betancourt J. L. (2012) Strontium isotopes and nutrient sourcing in a semi-arid woodland. *Geoderma* **190**, 574–584.
- Samuel, J., Rouault, R., Besnus, Y. (1985) Analyse multiélémentaire standardisée des matériaux géologiques en spectrométrie d'émission par plasma à couplage induc- tif. *Analisis* **13**, 312.
- Schreck E., Dappe V., Sarret G., Sobanska S., Nowak D., Nowak J., Stefaniak E. A., Magnin V., Ranieri V. and Dumat C. (2014) Foliar or root exposures to smelter particles: Consequences for lead compartmentalization and speciation in plant leaves. *Sci. Total Environ.* **476–477**, 667–676.
- Scotti I. A. and Carini F. (2000) Heavy metal effect on uptake and translocation of Cs and Sr in aubergine plants. *J. Environ. Radioact.* **48**, 183–190.
- Shalev N., Lazar B., Halicz L., Stein M., Gavrieli I., Sandler A. and Segal I. (2013) Strontium Isotope Fractionation in Soils and Pedogenic Processes. *Procedia Earth Planet. Sci.* **7**, 790–793.
- Šrek P., Hejzman M. and Kunzová E. (2010) Multivariate analysis of relationship between potato (*Solanum tuberosum* L.) yield, amount of applied elements, their concentrations in tubers and uptake in a long-term fertilizer experiment. *F. Crop. Res.* **118**, 183–193.
- Song B. Y., Gautam M. K., Ryu J. S., Lee D. and Lee K. S. (2015) Effects of bedrock on the chemical and Sr isotopic compositions of plants. *Environ. Earth Sci.* **74**, 829–837.
- Stewart B. W., Capo R. C. and Chadwick O. a (1998) Quantitative strontium isotope models for weathering, pedogenesis and biogeochemical cycling. *Geoderma* **82**, 173–195.
- Swoboda, S., Brunner, M., Boulyga, S.F., Galler, P., Horacek, M., Prohaska, T. (2008) Identification of Marchfeld asparagus using Sr isotope ratio measurements by MC-ICP-MS. *Anal Bioanal Chem.* **390**, 487–94.
- Takeda, A., Tsukada, H., Takaku, Y., Hisamatsu, S., Inaba, J., Nanzyo M. (2006) Extractability of major and trace elements from agricultural soils by chemical extraction methods: application for phytoavailability assessment. *Soil Sci. Plant Nutr* **52**, 406–417.



- Takeda A., Tsukada H., Takaku Y., Akata N. and Hisamatsu S. (2008) Plant induced changes in concentrations of caesium, strontium and uranium in soil solution with reference to major ions and dissolved organic matter. *J. Environ. Radioact.* **99**, 900–911.
- Techer I., Lancelot J., Descroix F. and Guyot B. (2011) About Sr isotopes in coffee “Bourbon Pointu” of the Reunion Island. *Food Chem.* **126**, 718–724.
- Tessier A., Campbell P. G. C. and Bisson M. (1979) Sequential Extraction Procedure for the Speciation of Particulate Trace Metals. *Anal. Chem.* **51**, 844–851.
- van der Heijden G., Legout A., Pollier B., Mareschal L., Turpault M. P., Ranger J. and Dambrine E. (2013) Assessing Mg and Ca depletion from broadleaf forest soils and potential causes - A case study in the Morvan Mountains. *For. Ecol. Manage.* **293**, 65–78.
- Vinciguerra V., Stevenson R., Pedneault K., Poirier A., Hélie J.-F. and Widory D. (2015) Strontium Isotope Characterization of Wines from the Quebec (Canada) Terroir. *Procedia Earth Planet. Sci.* **13**, 252–255.
- Vitousek P. M., Kennedy M. J., Derry L. A. and Chadwick O. A. (1999) Weathering versus atmospheric sources of strontium in ecosystems on young volcanic soils. *Oecologia* **121**, 255–259.
- Voerkelius S., Lorenz G. D., Rummel S., Quénel C. R., Heiss G., Baxter M., Brach-Papa C., Deters-Itzelsberger P., Hoelzl S., Hoogewerff J., Ponzevera E., Van Bockstaele M. and Ueckermann H. (2010) Strontium isotopic signatures of natural mineral waters, the reference to a simple geological map and its potential for authentication of food. *Food Chem.* **118**, 933–940.
- Whipkey C. E., Capo R. C., Chadwick O. A. and Stewart B. W. (2000) The importance of sea spray to the cation budget of a coastal Hawaiian soil: a strontium isotope approach. *Chem. Geol.* **168**, 37–48.

**Figure caption**

**Figure 1:** Location of “The Costières Mill” and Serfel sites in southeastern France with a satellite map detailing the emplacements of the cultivated plots. The geological context of the studied area is also provided.

**Figure 2:** (a) Detailed sketch of the experimental plots of the olive trees at the Serfel site: three repetitions (Rep1, Rep2, Rep3) of three irrigation modalities (no irrigation, moderate irrigation and high irrigation) were studied. The plots  $\Phi$ , B and D were selected with six olive trees sampled in each repetition for Sr measurements. (b) Mapping of the <2mm fraction and of detected carbonate in the soils of the Serfel plots.

**Figure 3:** (a) Boxplot presentation of  $^{87}\text{Sr}/^{86}\text{Sr}$  of branches and leaves sampled on the Serfel and “The Costières Mill” sites; (b) Boxplot presentation of  $^{87}\text{Sr}/^{86}\text{Sr}$  of combined ‘branches + leaves’ samples according to the irrigation modalities. The long central rectangles of the boxes represent 75% of their contents with a transversal bar for the median value of these data, the highest and lowest values being identified by the circles above and below.

**Figure 4:** (a) Boxplot presentation of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from mobile and exchangeable fractions of soils according to the sites and the irrigation modalities. Plots of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of (b) the mobile and (c) the exchangeable fractions of soils from “The Costières Mill” and the Serfel sites as a function of depth.

**Figure 5:** Relationship between the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of all studied reservoirs as a function of a vertical profile (oil data is from Medini and *al.*, 2015).

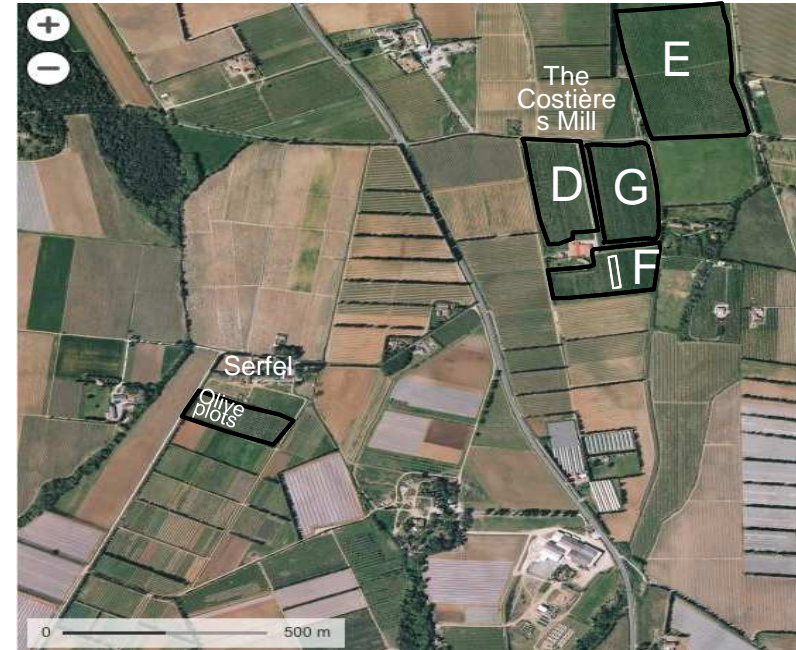
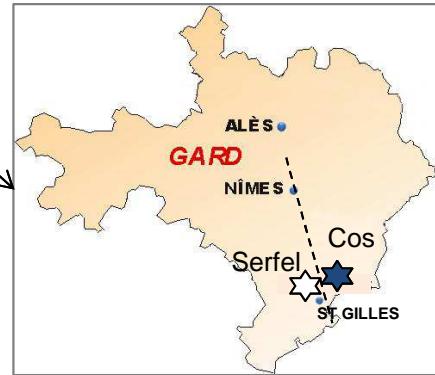
**Figure 6:** Mixing diagram  $^{87}\text{Sr}/^{86}\text{Sr} = f(\text{Sr})$  showing relationship between soil samples and plant-protection products.

## Supporting Material

**Table 1:** Sr concentrations and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of branches and leaves from Picholine and Aglandau trees sampled at the Serfel and “The Costières Mill” sites.

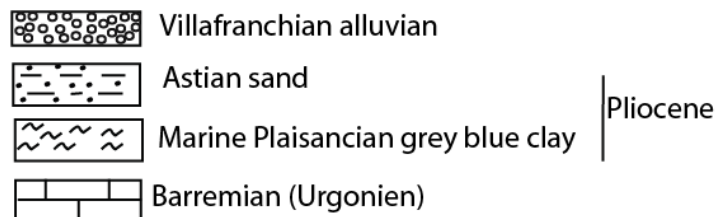
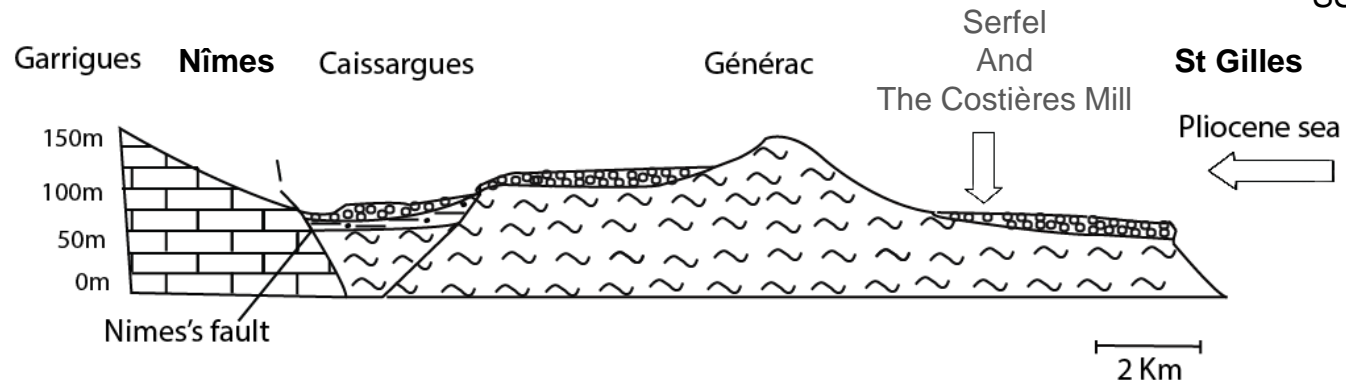
**Table 2:** Sr concentrations and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the mobile and exchangeable fractions of soils collected at the Serfel and “The Costières Mill” plots.

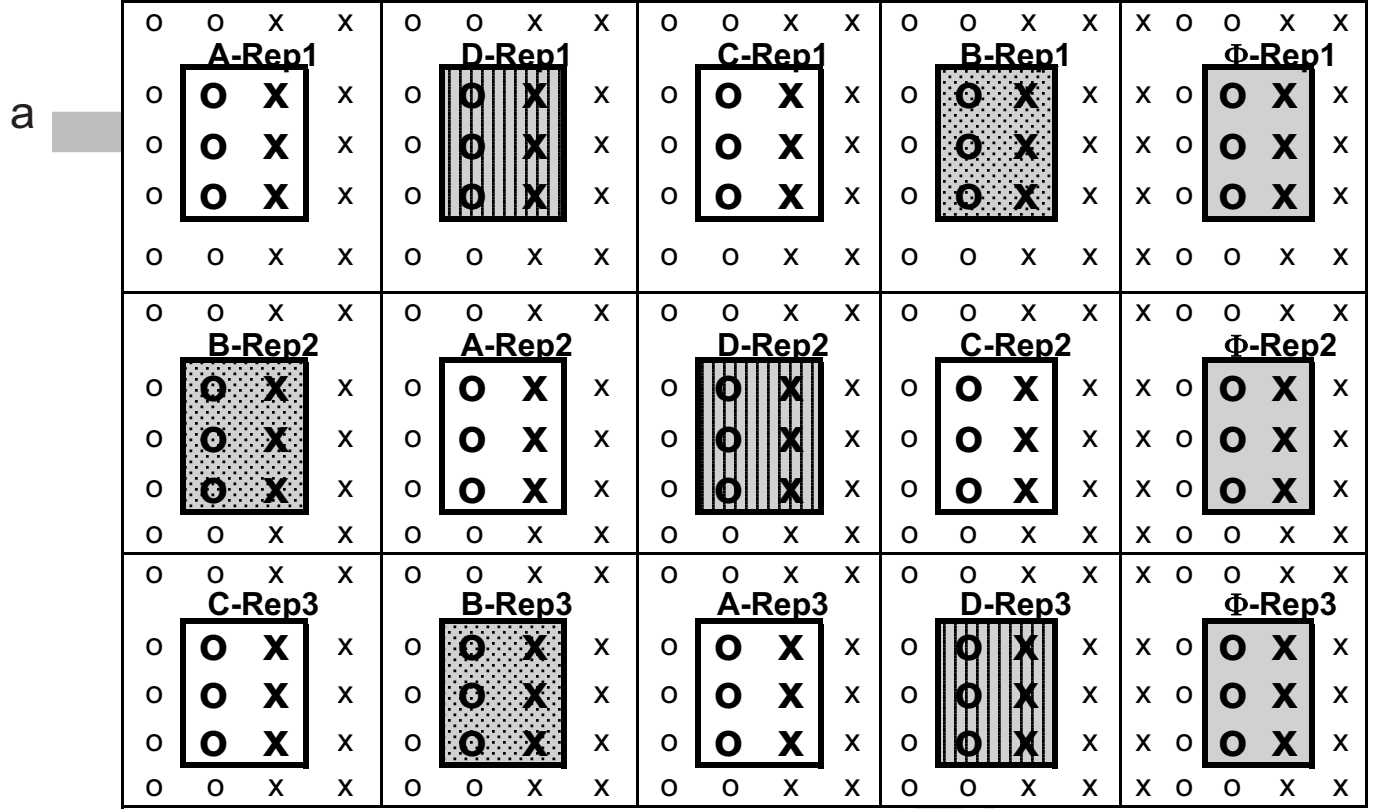
**Table 3:**  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the plant-protection products and fertilizers as whole products (WR) and leachates (SP), and of waters (rainwater and irrigation water).



NNW

SSE



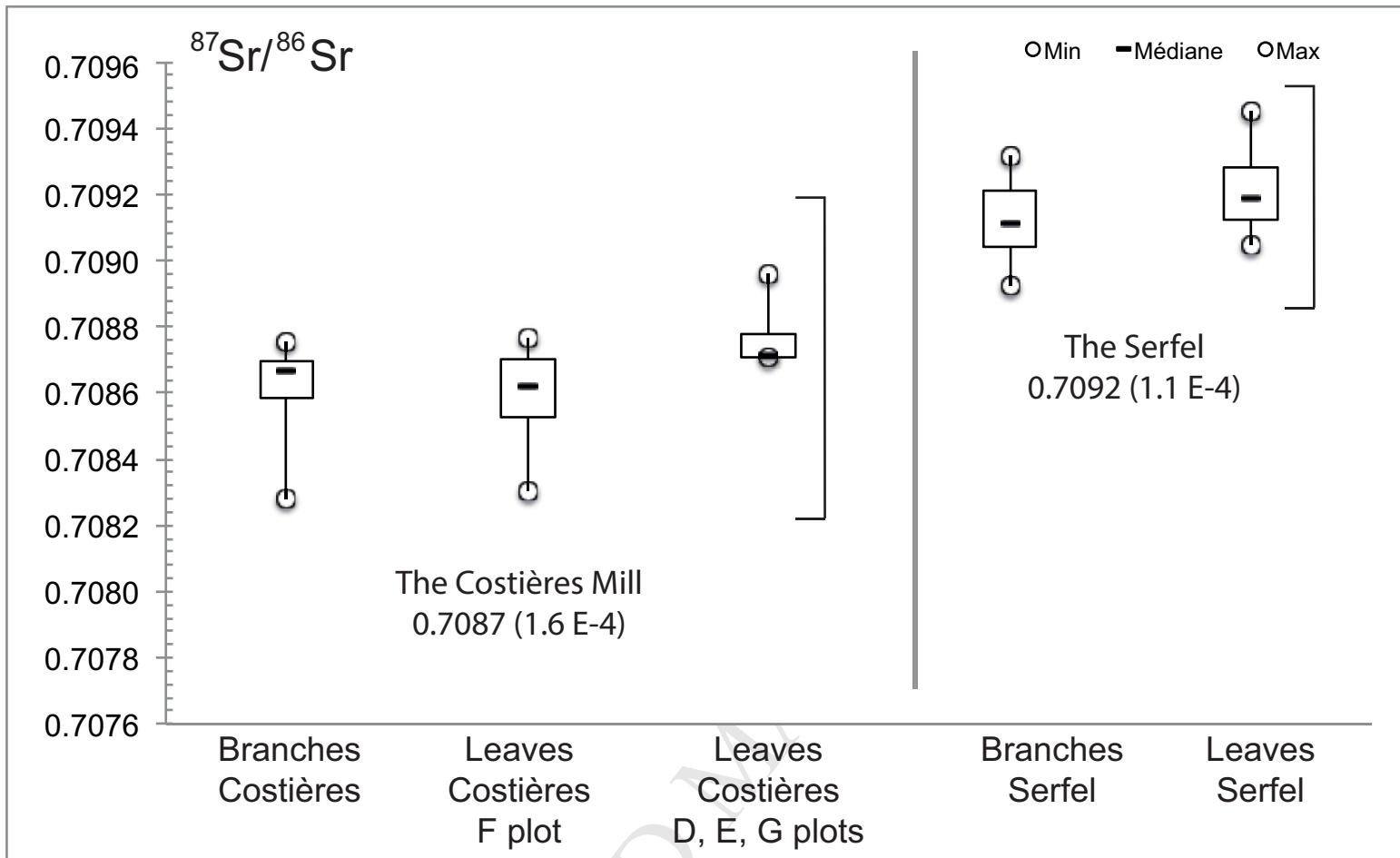


- Selected plots for this study
- Aglandau trees at the edge
  - Aglandau trees selected for analyses
  - x Picholine trees at the edge
  - X Picholine trees selected for analyses
  - Not irrigated plots ( $\phi$  groups)
  - Moderate irrigation (D groups)
  - High irrigation (B groups)

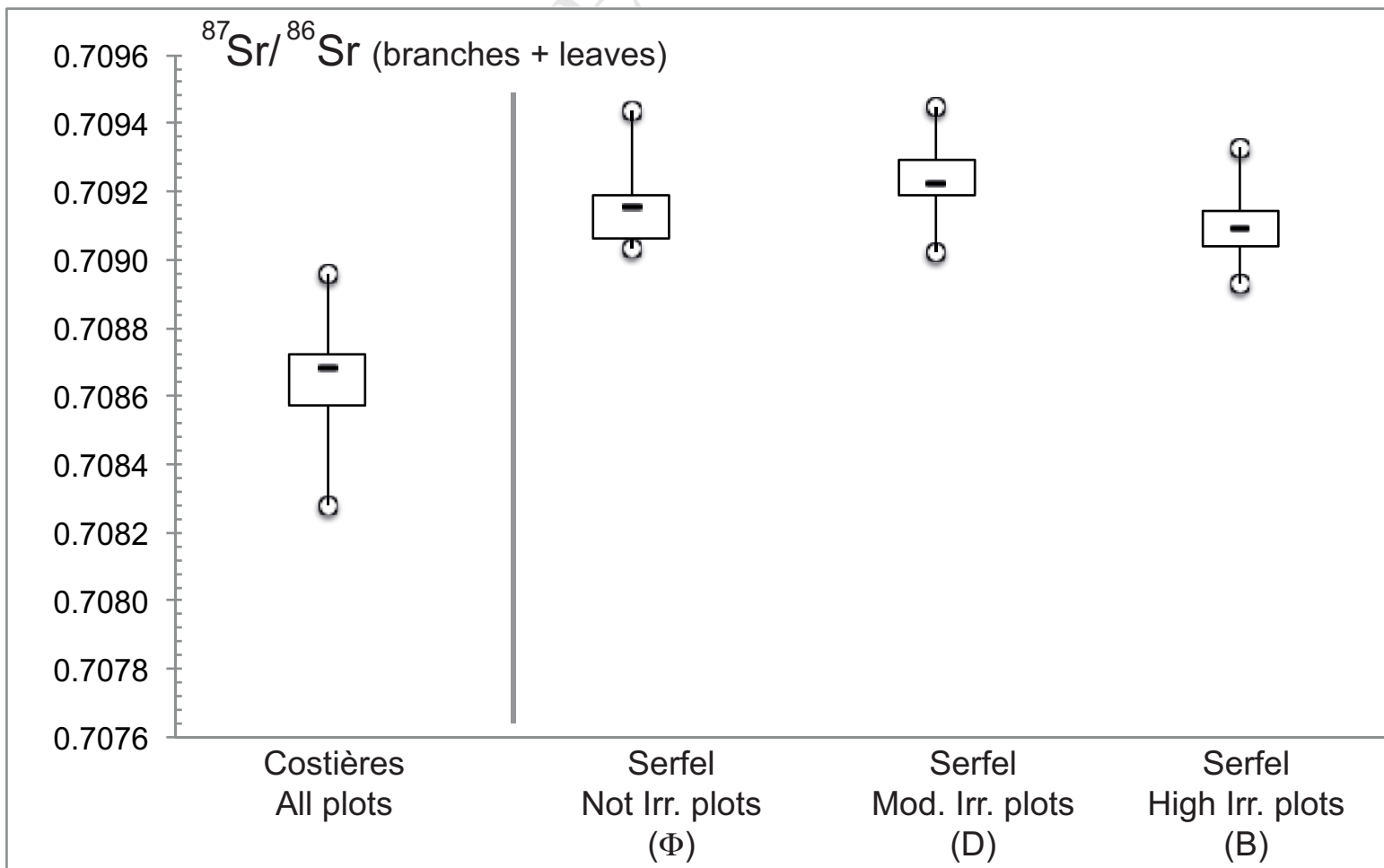


- Less than 70% in the deep profile
- More than 70% in the 0-25 cm zone
- More than 70% in the 0-50 cm zone
- More than 70% in the 0-75 cm zone
- More than 70% in the 0-100 cm zone
- Carbonates free zone
- Occurrence of carbonates

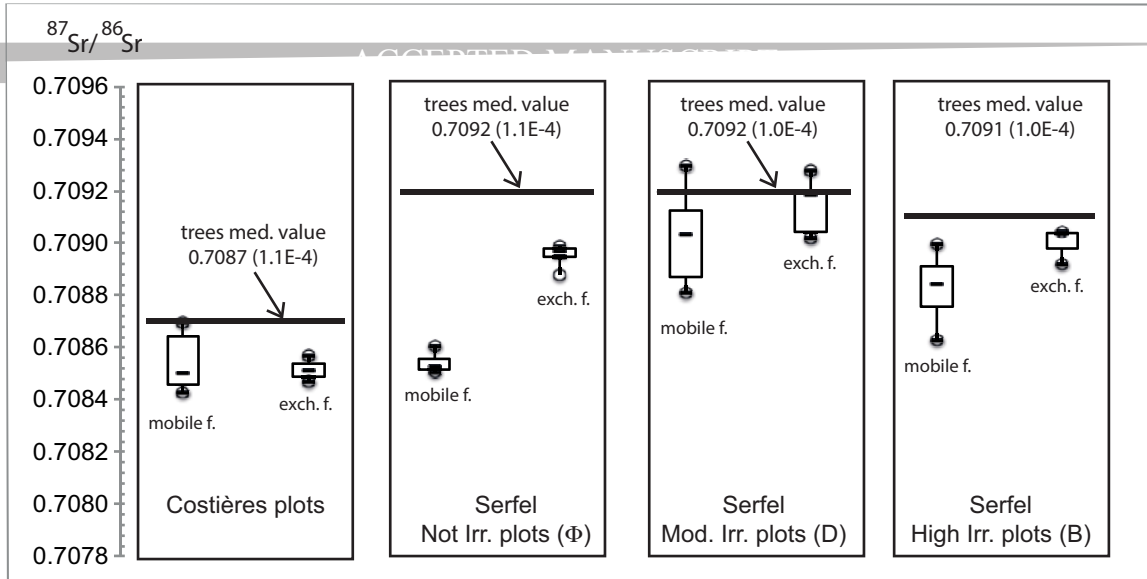
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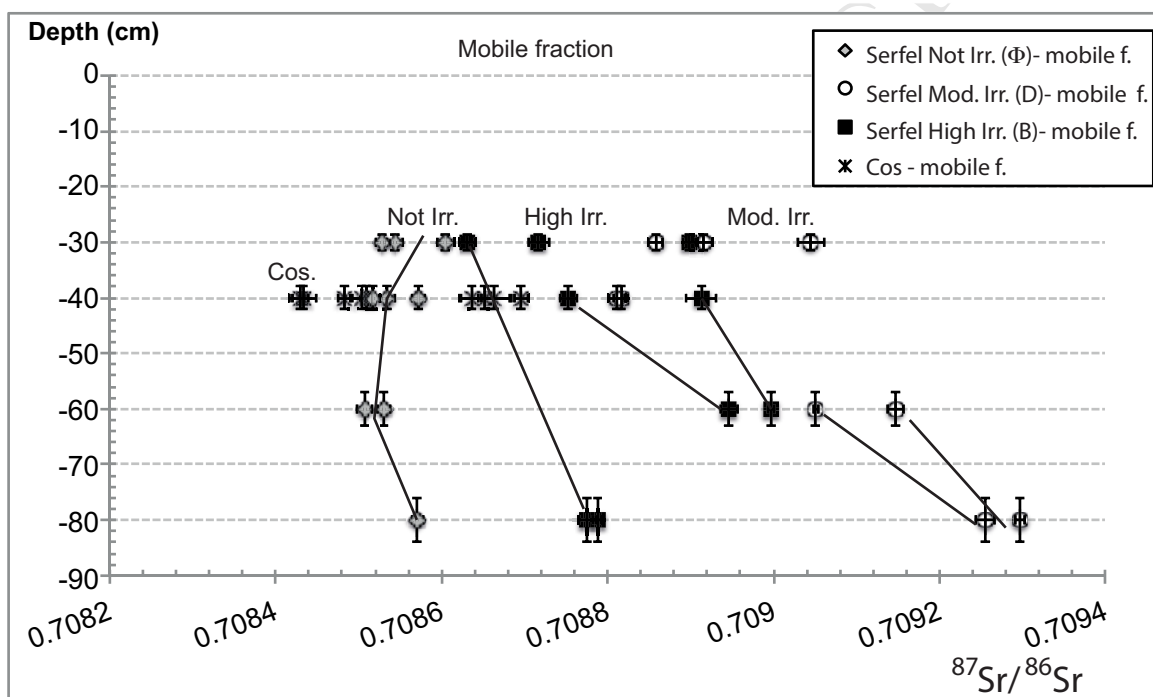
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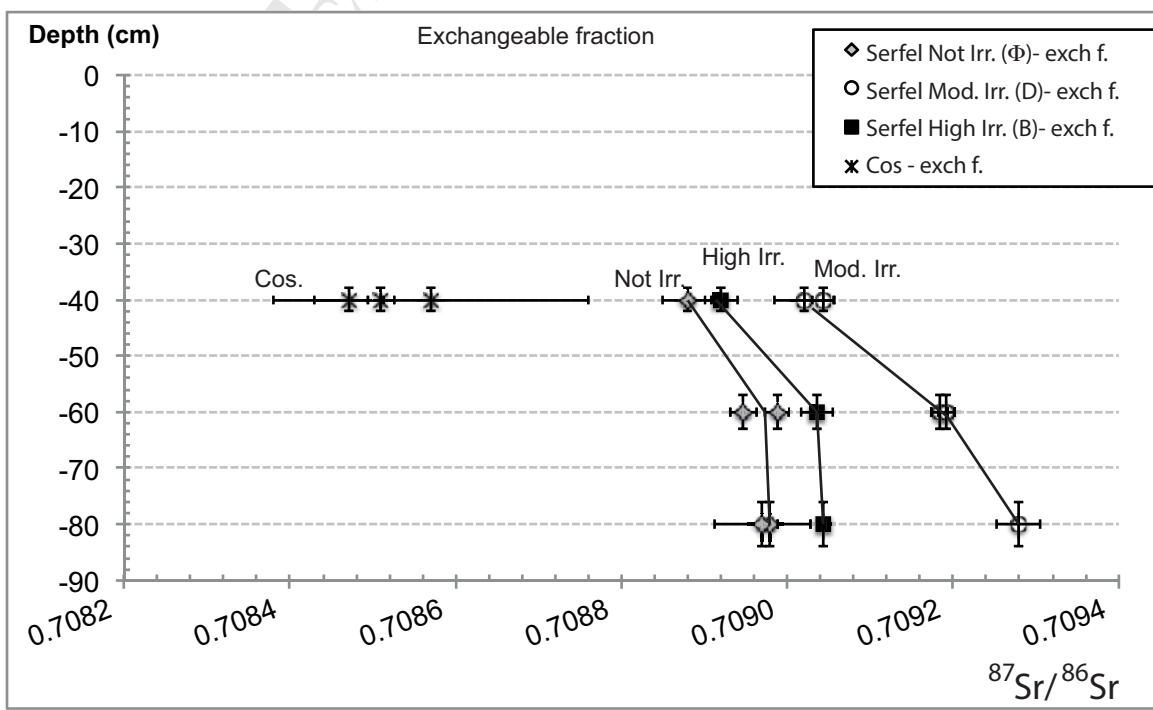
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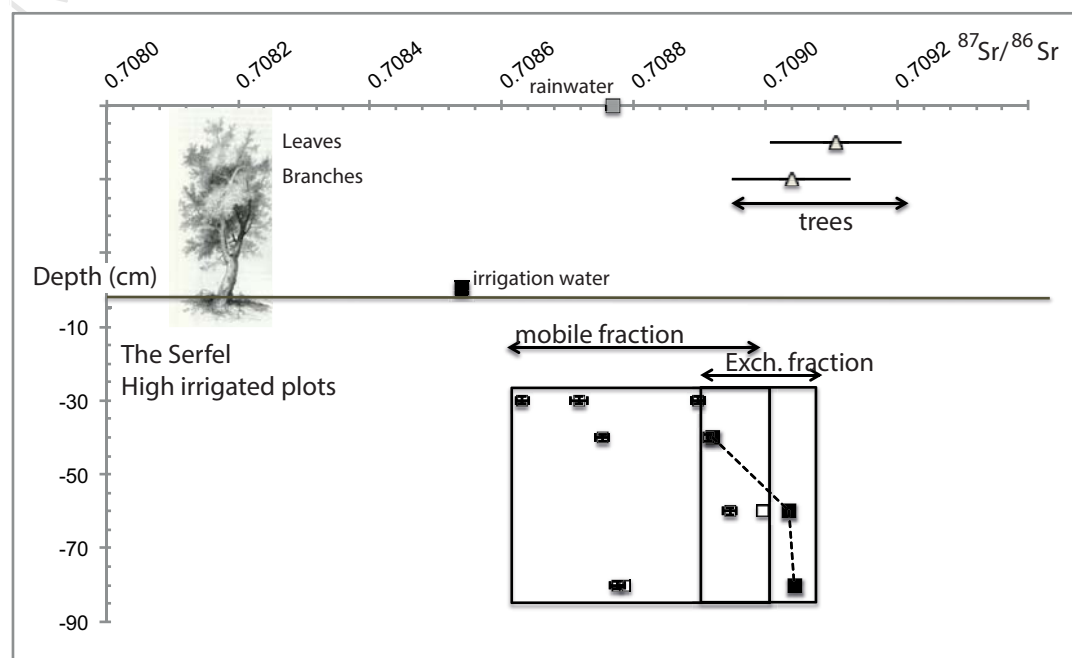
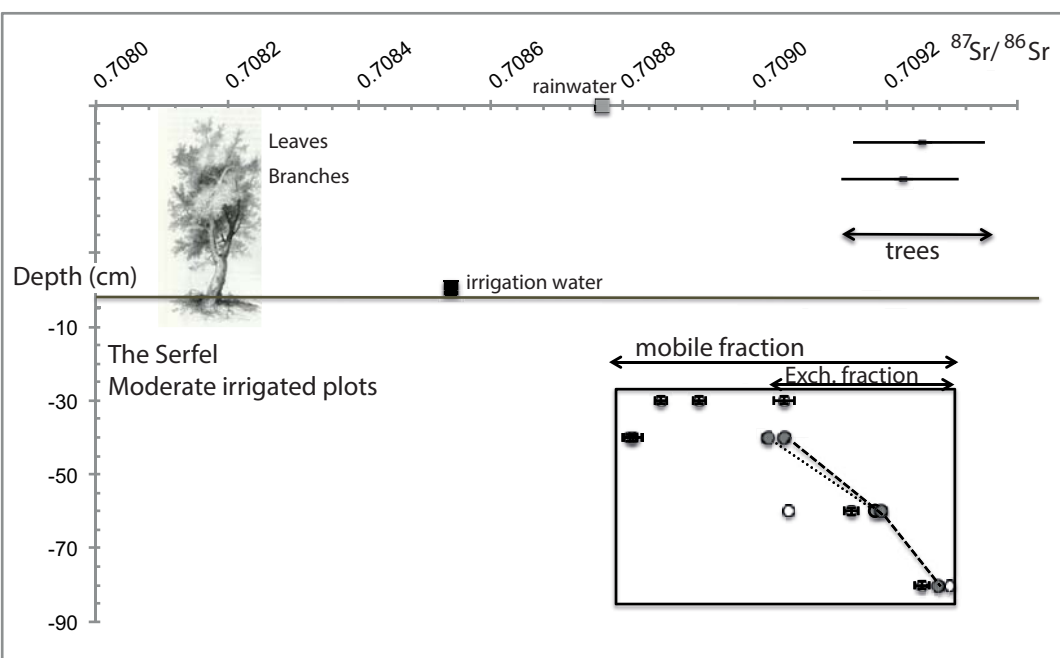
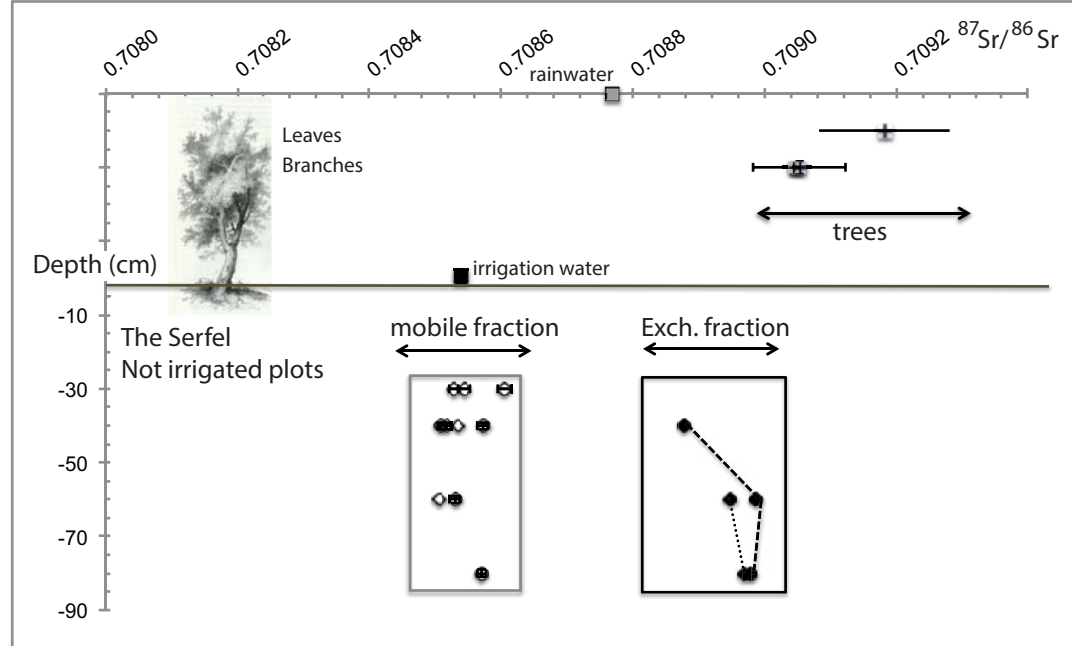
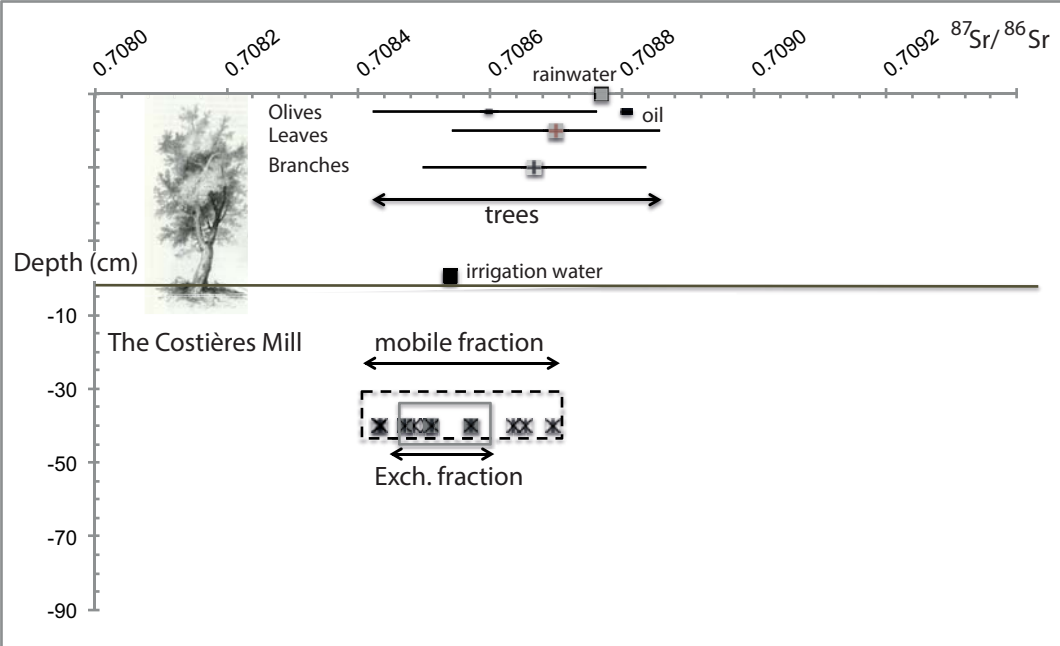


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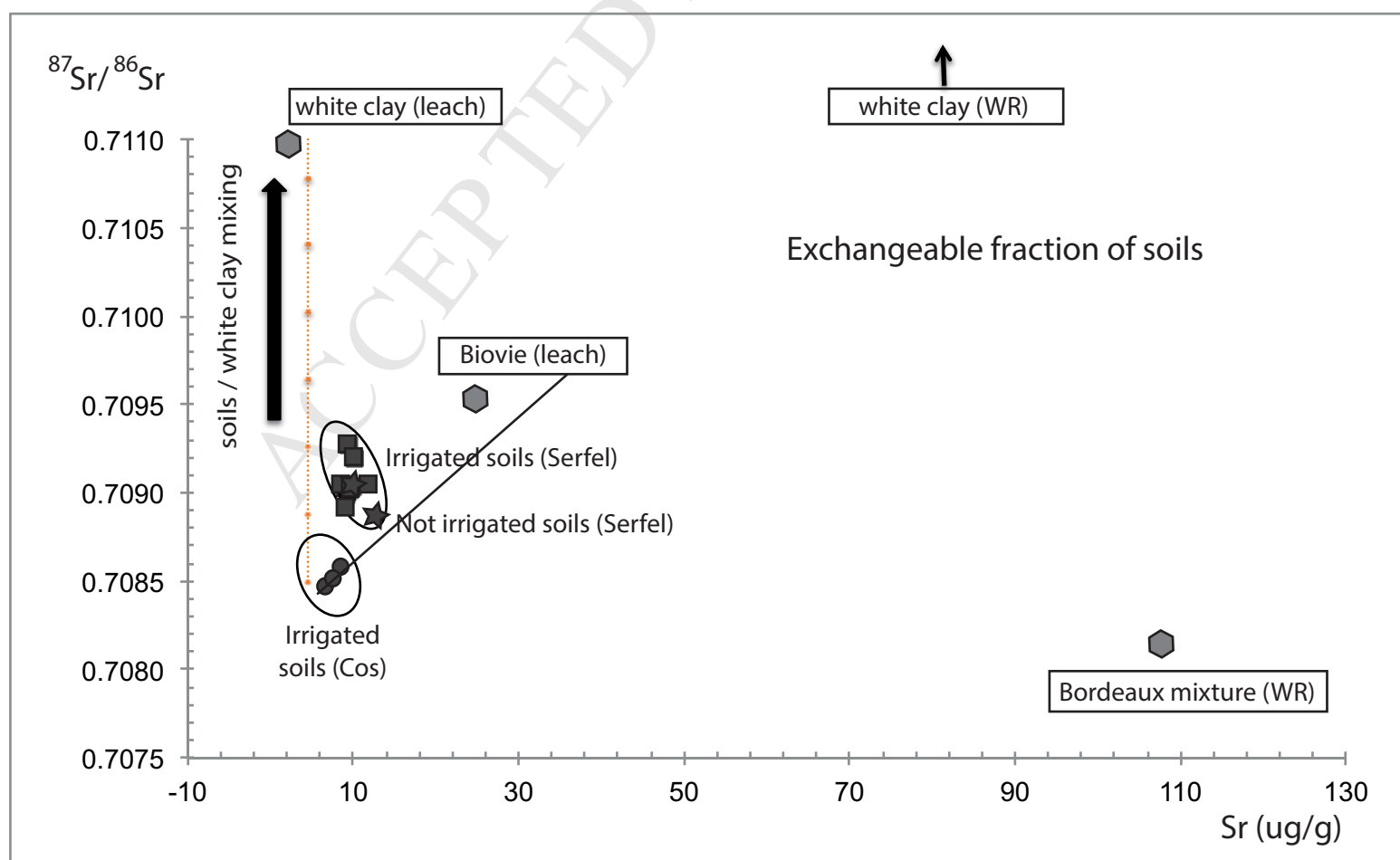
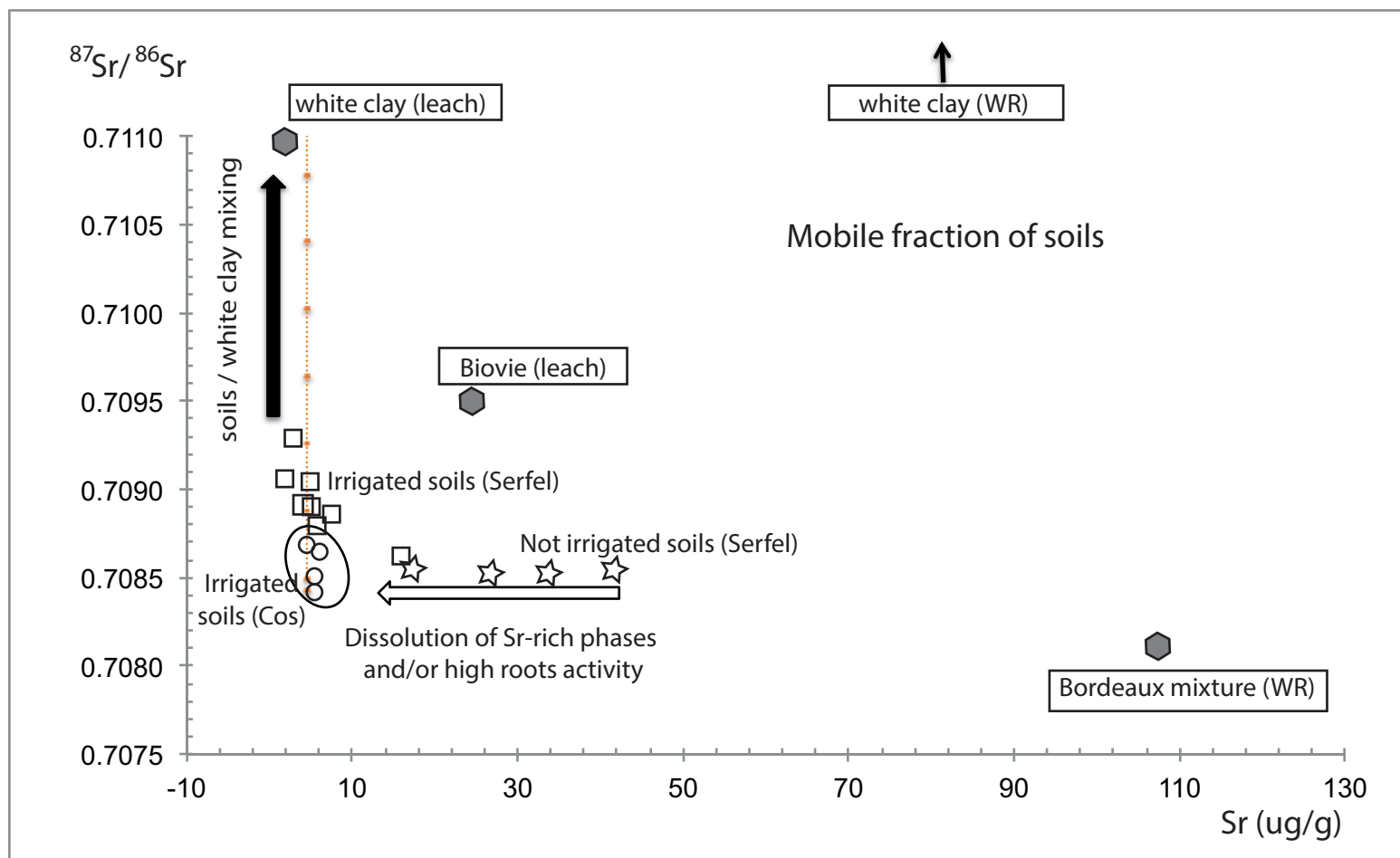


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ACCEPTED MANUSCRIPT

# **Impact of agricultural practice on the Sr isotopic composition of food products: Application to discriminate the geographic origin of olives and olive oil.**

## Highlights:

The origin of Sr in trees in agricultural contexts is discussed

Irrigation and fertilization have significant effect on trees Sr isotope ratio

Sr isotope could be used as tracer of the geographic origin of agri-food products

The efficiency of the tracing tool is strictly dependent on agricultural modalities