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"Application of a multidisciplinary approach to investigate on a possible secondary soil salinization, due to seawater intrusion dynamics, at the Volturno river mouth"

Scientific sector: AGR/05

Coordinator: Prof. Paolo De Angelis

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Corso di Dottorato di Ricerca in Ecologia Forestale - XXVIII ciclo
Coordinatore: Prof. Paolo De Angelis

Tesi di Dottorato di Ricerca in Ecologia Forestale
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In memoria di mia mamma

Rosanna

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Table of contents

Organization of the Thesis

This PhD thesis consists of six main sections.

A brief introduction to the studied phenomenon and its causes/consequences is provided in the first part. Also, the main characteristics of the pilot study are presented. A following subsection highlights the differences between primary and secondary salinization. Then, the chosen approach was discussed, pointing out its advantages on such a study. Lastly, we examine the context in which the project was born, its origin and objectives to pursue.

In the second section, we review the features of the study area from the socio-economic, political, environmental and geo-morphological points of view.

The third section is about the techniques and methodologies founding the mentioned multidisciplinary approach, showing the samplings and measuring phases in detail.

The overall results are presented in the fourth section, referring to each source methodology.

In the fifth section, the results were deeply discussed, and compared to previous, similar case studies.

Lastly, in the sixth section, we illustrate the conclusions of the pilot study, giving proposals and suggestions for the next steps.

Beside those six main sections, other specific sections were used for abstract, bibliography, tables and figures sources list. Finally, a personal publications and contributions list is shown, giving a brief summary of the different study fields in which the PhD student has been involved.

Abstract

The coastal areas are characterized by high population rates and vivid economic activity. This involves a continuous increasing of water demand, often associated with a non-sustainable exploitation of the water resource.

For this reason, frequently, groundwater located near the coast are compromised by the saltwater intrusion.

In this PhD thesis, we would like to introduce a pilot study for investigating on a possible secondary salinization at the Volturno river mouth. The study comes as a result to reply to the testimonies of the local population, who reported an impairment of water quality and soil, consequently.

In our study area, we are in presence of severe pumping, which is mainly due to the urban and agricultural activity. Furthermore, thanks to previous research carried out in the same study area, a lowering of the river bed relative to sea level has been proved. These two elements suggest that salinization could be favored by up-coning phenomenon and lateral ingression through the river bed.

As a first step, site investigations were carried out, including inspections to choose the study area, interviews with local people (in order to get information not available from published sources) and photographic reports.

The second step was designing an experimental study - based on a multidisciplinary approach - in order to better address a very complex topic such as the salinization due to seawater intrusion.

Along a chosen transect, crossing three different ecosystems, with different degrees of naturalness (coastal, urban and agricultural), several measurements and samplings were carried out, and the elm tree species (*Ulmus minor* Mill.) was selected as bio-indicator. The multidisciplinary approach is based on:

- analysis of carbon and oxygen stable isotope compositions and discrimination,
- leaf gas exchange measurements on elm,
- normalized difference vegetation index (NDVI) calculations;
- geophysical investigations.

Such a composite approach revealed a rather complex system along the riverine transect.

Since this is a pilot study, which has clashed with practical problems due to the lack of cooperation from part of citizens and local authorities as well, the reported data are meant to be preliminary. Preliminary results did not show any salinization emergency but proved salt presence in some of the ecosystem compartments, suggesting, in addition, that saltwater presence seems to be due to both a lateral intrusion through the river's meanders and to an improper management of water and irrigation practices. Despite all, they do not allow to discriminate which is the main phenomenon regulating the entire salinization process.

Our analyses indicate that water enriched with the heavier oxygen isotope affects photosynthesis, stomatal aperture and electron transport efficiency. In particular, the decrease of photosynthesis in the rural area suggests that the different land-uses play an important role.

Furthermore, high resolution ERTs confirmed salt water's presence into the groundwater.

The initial phase of this pilot study confirmed the validity and potential of our approach and, at the same time, have shown the need to plan monitoring activities in the Volturno coastal area. Of course, additional measurements campaigns and experiments will be required; in that regard, we listed some proposals for a monitoring study (reported at the end of this work), which will also be presented to the competent authorities.

Another essential question is the need to inform and educate the citizens to adopt sustainable management of land and resources, remarking that the salinization hazard is a serious threat to the economy.

The study was part of research activities promoted by high technology infrastructure for environmental and climatic monitoring (PON I-AMICA).

1 Introduction

Among the most discussed environmental issues in recent decades, compromising of water quality resources plays a major role. Scientific research has focused particularly on salinization of aquifers, paying attention to the different contexts and the different possible pollution sources, discriminating the contribution of natural factors and the role of human activities.

FAO (2005 http://www.fao.org/ag/agl/agll/spush) data, reported by Munns (2005) and several other authors, showed that over 800 million hectares of land (6% of the world's total land area) are salt-affected, either by salinity (397 million ha) or sodicity (434 million ha). In addition to natural causes, a fundamental role is played by irrigation practices. Actually, about 2% of dryland agriculture land and 20% of irrigated agriculture land are salt-affected (FAO, 2005).

Even in Europe the situation is very serious, where soil salinity has effects on one million hectares, mainly in the Mediterranean countries (Thot *et al*., 2008). This phenomenon increases in proximity of coastal areas, which have high rates of population density and, consequently, higher demand for both drinking and irrigation water.

In addition to the coastline, special attention should be paid to the "transition environments", such as brackish lagoons and wetlands, located between the coast and the backside environments, like forests or agricultural fields, which often have great ecological and economic value. The groundwater of the entire zone, which includes the coastal, transition and surrounding environments, are particularly sensitive to salt water intrusion. This phenomenon is due to the increase of the salt-wedge, i.e. the advancement of the natural interface between fresh water of a coastal aquifer water table and salt water of the adjacent sea (Figure 1). Due to the different densities, at the transition zone between fresh and salt water (described as a net interface for simplicity), the sea water settles on the bottom and is wedged under the fresh water. This phenomenon, which is entirely natural, is due to the increasing action of natural and anthropogenic forcing (lowering of the water surface level and excessive water pumping from wells, respectively) might speed up drastically, causing a hydraulic imbalance that determines the salt water feed.

The density flow is associated not only with the density itself, but also with parameters such as temperature, salinity concentration or both, simultaneously. Even the water saturation ratio of porous media changes spatially and temporally, and influences the aquifer parameters, affecting the pressure gradients of the flow. In literature, there are many publications whose aim is trying to understand the complex physical processes regulating the variable density flow. Nevertheless, not all issues involved have been clarified.

In most of the cases, salt water intrusion is caused by excessive pumping from water wells, responsible of saltwater upconing, described by Reilly and Goodman (1987) as the movement of saltwater from a deeper saltwater zone into the fresh groundwater in response to the pumping. Among the factors that are worth mentioning, we can find: the rate of water extraction, the number of wells, the closeness between the wells and the proximity of the wells to the coast. Definitely, the excessive water pumping determines a rise of salt/sea water and the movement of saltwater/freshwater interface, causing a damage in groundwater quality with serious economic, social and environmental consequences (Chaves *et al*., 2009).

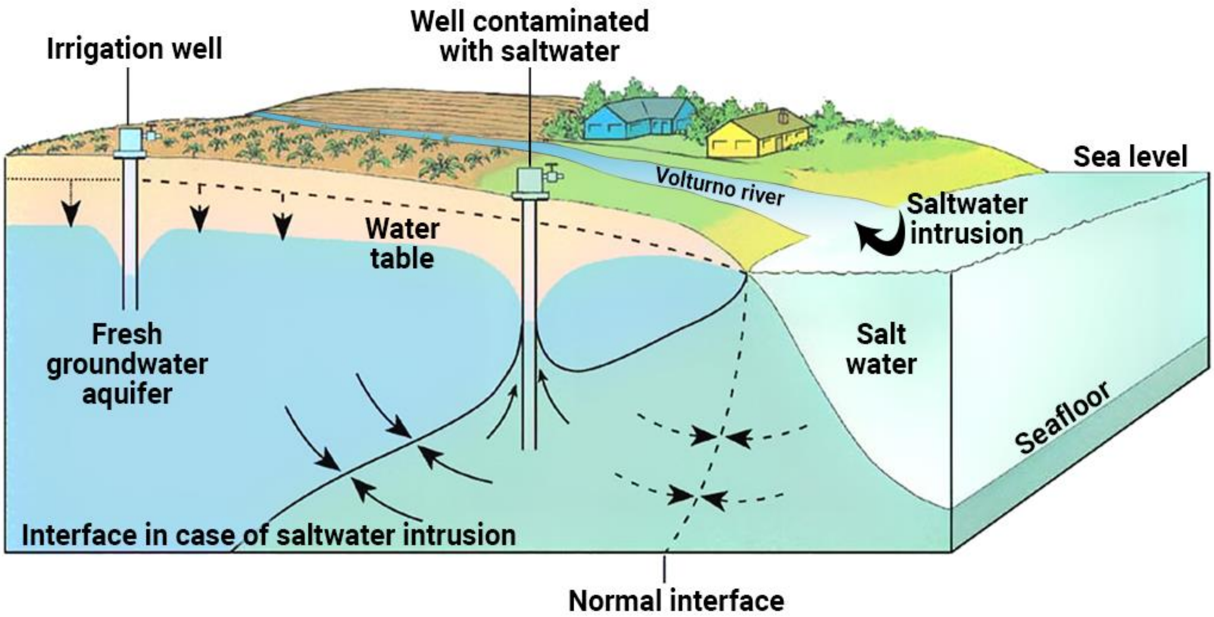

Figure 1: Graphical representation of saltwater intrusion phenomenon in groundwater near Volturno River’s mouth.

The first issue to address is the degradation of the aquifer quality. Since groundwater is the main source of drinking and irrigation water, its deterioration forces to find alternative water supplies which are often expensive (Cheng *et al*., 2000).

Another important point to consider is the reduction of crops and pastures production, which impacts the sale of those goods both directly and indirectly, by interfering with livestock nutrition, as well.

Ultimately, reducing soil quality and vegetation cover, salinization causes the loss of soil fertility, altering the soil structure and exposing it to erosion, with a desertification risk which gets even higher for arid, semi-arid and sub-humid ecosystems. This results in a substantial loss of territory that may reach alarming levels when acting in combination with other agents such as weather; that is particularly true close to the coasts and river mouths.

The salinity effects on vegetation depends on various factors such as species, variety, growth stage, environmental factors and nature of the salts (Yadav *et al.*, 2011). Physiologically, vegetation tries to implement adaptation and tolerance processes, in order to respond to the salinization phenomenon; however, generally speaking, a deterioration of the physiological state is almost inevitable. Plants govern water movement in the soil by osmosis, which is controlled by the level of salts in the soil water and in the water contained in the plant. If the level of salts in the soil water is too high, water may flow from the plant roots back into the soil. This results in dehydration of the plant, causing yield decline or even death of the plant. Monitoring its responses allows to indirectly control the situation in place, trying to identify possible intervention strategies.

It is clear that the environmental and the economic contexts are interwoven. The economic side is definitely one of the most alarming to farmers, business owners and local governments, but it is not the only nor the most important one.

In fact, the perspective that should raise more interest (and concern, at the same time) is the ecological one - especially in presence of a natural environment characterized by a high biological index, hosting a wide biodiversity of *flora* and *fauna* and subject to strong anthropic pressure that results in the need of implementing different degrees of protection.

This pilot study was planned in response to complaints from some local farmers, who noticed a progressive water/soil salinization and, consequently, a decrease in their fields' productivity. It is based on the plants' physiological responses to certain environmental conditions and on the analyses of soil and ground water quality. The ecophysiological measures are also coupled with satellite and geophysical investigations, in order to obtain a more complete analysis of the problem in place. The purpose is to bring attention on a delicate matter, especially in an area that is already subject to different anthropogenic forcings. Hopefully, a proper monitoring study could be born from this preliminary study. Actually, bio-monitoring coastal zones becomes a useful tool to investigate the current situation and see if a salt water ingression into the aquifer is occurring (or is likely to occur), and what ecophysiological responses have been implemented by the plants.

Therefore, the salinization and saltwater intrusion phenomenon will not be discussed from the mathematical-analytical/physical point of view.

Among the long-term objectives, there is the promotion of better management of water resources, optimizing the pumping rates. This will be possible only when the entire population will be aware of the issue and educated to sustainable management. In this way, in addition to addressing and limiting a dangerous and damaging phenomenon for the environment, they will also have benefits in economy and productivity.

1.1 Primary and secondary salinization

Salinization of aquifer (and, consequently, of soil) is the process by which a non-saline resource becomes saline, by natural factors or as a result of resources mismanagement by humans.

The process begins when a transition zone between fresh and salt water is degenerated due to the seawater flows that are introduced in the fresh aquifer. Because of the capillary rise and/or the pumping for irrigation purposes, the water with a higher concentration of salts will penetrate and soak the soil, causing its salinization.

When ground water salinization is due to natural factors, it is called "primary salinization", while it is defined "secondary salinization" when due to anthropogenic factors (Tóth *et al*., 2008).

Among the natural factors, we must mention the occurrence of natural geological, hydrological and pedological phenomena, which are essentially based on chemical and physical processes, responsible of rocks decomposition and disintegration. Other factors that may contribute to an increase in salts concentration are: the presence of channels - which may cause the rise of salt groundwater to the surface - and the presence of micro-depressions with little or no drainage (http://eusoils.jrc.ec.europa.eu). Furthermore, especially in coastal areas, the wind action, the sea salt aerosol presence and the occurrence of tides, contributes to the salinization problem. Despite being the main causes of the salinization phenomenon, those factors are homogeneously distributed in time (geological time) and space (the entire globe), coming fully in the natural dynamics.

This natural balance is easily broken when anthropogenic forcing impacts; even though it is composed by infinitely smaller entities, they are concentrated in space and time.

Land use patterns strongly influences the salinization process as showed in the European Soil Bureau report (Eckelmann *et al*.,2006), where salinization is defined as one of the major soil threats along with organic matter decline, erosion, compaction and landslides. Among the various anthropogenic-originated causes, the main responsible is the improper management of water and irrigation methods, like irrigation with waters rich in salts, improper or poor irrigation practices, inadequate drainage, utilization of fertilizers and other inputs. Szaboles (1992) estimated that 50% of all irrigated schemes are salt affected, and generally the situation is worsening due to the increasing water demand for domestic, industrial and agricultural purposes (FAO 2014 www.fao.org/nr/aquastat).

1.2 Multidisciplinary approach

Being the sea water intrusion a complex process, that depends on several aspects like seasonal conditions and interactions with groundwater (Rengasamy 2006), land use patterns, natural geological, hydrological and pedological processes and human activity in the last years, a number of multidisciplinary studies have been carried out to investigate this phenomenon and its effects on vegetation (Zhang and Dai, 2001; Zakhem and Hafez, 2007; Chitea *et al*.,2011; Arsalan *et al*.,2012; Singh *et al*.,2013).

These kinds of researches are usually addressed to ecophysiological, chemical or geophysical investigations separately. However, there are few studies involving different disciplines. Hence the idea of supporting the ecophysiological measurements (isotopic analysis and gas exchange measurements) with geoelectric prospecting, satellite and photographic surveys, and with the testimonies of the locals. Even though this is a pilot study, it is indeed innovative and interesting, especially given the context in which it takes place.

Actually, this study was initially meant to be developed into an actual monitoring activity. In that perspective, several sampling campaigns were considered, in order to monitor chemical, physical and – especially - isotopic water composition. Moreover, the digging of twenty new wells was scheduled, in order to monitor the aquifer's water quality through a multi-parameter probe and a targeted sampling. Unfortunately, due to budget issues related to the project and to organizational problems mainly due to difficulties encountered in the study area, it was necessary to downsize the project. However, this initial version of our study allows to show its full potential, and how important it is to focus on this type of approach. Soon a proposal to extend this study will be presented, following the initial idea, in order to start a real monitoring project at its fullest.

1.3 Project

The study was supported by high technology infrastructure for environmental and climatic monitoring (PON I-AMICA) coordinated by National Research Council of Italy (CNR) and funded by Italian Ministry of Education, University and Research (MIUR) under the National Operative Programme (PON). The study is part of OR4: "Technological applications and territorial services". These applications are devoted to identify, define and quantify the contributions that natural and anthropic processes have in affecting the environmental-climatic conditions in the Italian Convergence Regions, and they are focused on land-water interactions, in order to solve important problems such as the introgression of the salt wedge affecting the lives of agro-forest ecosystems, especially near the coasts.

This work meets the expectations of the first phase of the I-AMICA project. That is setting up a research plan, identifying an innovative and multidisciplinary approach and carrying out the pilot analysis in order to determine whether the chosen methodology meets the defined objectives.

To that end, in this PhD thesis we present a complete territorial framework, along with the used methodologies and approach, and the preliminary research results that will be discussed on other similar studies.

2 Characterization of the study area

2.1 Socio-economic context

The " Rapporto sullo Stato dell'Ambiente di Castel Volturno 2009" provides a quite detailed snapshot of the area and its environment (http://www.impronte.altervista.org/alterpages/files/RSACASTELVOLTURNO.pdf).

The Castel Volturno municipality is located on the left bank of the Volturno River, on its last bend before flowing into the Tyrrhenian Sea, with 25 km of beach. It covers a 72.23 km^2 area in the province of Caserta, and it is bordered by the municipalities of Cancello Arnone (CE), Mondragone (CE), Villa Literno (CE) and Giugliano in Campania (NA).

The resident population has about 23,870 inhabitants (DEMOISTAT, 2010 www.demo.istat.it) with a population density of 330 inhabitants / km^2, higher than the national average and lower than the provincial and regional media. The analysis of historical data shows an exponential increase of the resident population starting from the Second World War. In the last decade, this increase in population is mainly due to migration from other towns and foreign countries. According to the last census, citizens of foreign countries are about 2512, mainly originating from African nations (DEMO ISTAT, 2010). The population shows an unemployment rate significantly higher than the provincial, regionally and nationally trend.

Overall, the economic activity of the municipality is divided into: commerce (29%), agriculture (23%), industrial (15%) and other services 21% (ISTAT 2001). The trade and "other services" sectors are extremely variegated and mainly characterized by small activities. There are 242 farms in the territory, with a clear prevalence of areas intended for vegetable crops, and in particular for tomatoes cultivation. The livestock sector is very important as well, with around 148 companies (ISTAT, Agriculture Census 2000 www.istat.it) and more than 14 thousand heads of cattle, mainly buffalos and bovines. The industries are primarily related to construction and manufacturing sectors, although the zone has a quite remarkable dairy vocation.

2.2 Political context

The political situation of the Castel Volturno municipality is very unstable and often intertwined with mafia associations. In fact, in April 2012, the administration of Castel Volturno was suspended by competent authorities, because it was proved that organized crime conditioned the political and administrative decisions. Due to such instability, even the elections of June 2012 were suspended. This situation went on for about two years, until new democratic elections took place in June 2014.

It’s worth mentioning that Castel Volturno is not far away from the so-called "Land of Fires", illegally used by corrupt Italian politicians and businessmen as a dump for toxic substances. The history of this place, together with high unemployment and immigration levels, makes it a very difficult area, in which local crime meets and clashes with other types of crime, especially Nigerian mafia. As a result, an impressively large socio-cultural degradation is going on, in which the weaker population is continuously overwhelmed.

2.3 Environmental context

Although it is subjected to various forms of degradation, the Castel Volturno area has a high biodiversity index, and features considerably interesting characteristics from that point of view. Therefore, establishing different degrees of protection was necessary.

Protected areas insisting on the Castel Volturno territory are:

- the national-interest site of "Litorale Domitio Flegreo ed Agro Aversano", which includes the territories of 77 municipalities belonging to the provinces of Naples and Caserta;

- Regional Natural Reserve “Foce Volturno / Costa di Licola / Lago Falciano”, established by DPGR 12 February 1999 n. 65 (http://www.riservevolturnolicolafalciano.it), which protects a large area, between the cities of Castel Volturno and Giugliano in Campania and includes the estuary of the Volturno river, the basin of the Patria lake, and the sandy coastline stretching between these. The reserve covers an area of approximately 1,540 ha and includes several important areas subject to protection: the Variconi Oasis (about 60 ha), the State Natural Reserve ‘Castelvolturno’ (268 ha), the “SIC IT8010020 Pineta di Castel Volturno” (90 ha), the “SIC IT8010021 Pineta di Patria” (313 ha), the

“SIC -IT8030018 Lago Patria” (507 ha) and the “SIC IT8010028 Foce Volturno – Variconi” (303 ha) which contains, in turn, the “ZPS-Variconi IT8010018” (194 ha).

The Variconi Oasis (established in 1978), in which part of our study area lies, plays an important role: located on the left bank of Volturno’s mouth, it is characterized by the presence of coastal ponds that constitute the last river estuaries wetlands left in Campania, protected by the Ramsar Convention. The area is an important resting, wintering and nesting site for birds, as witnessed by a group of volunteers who have created an association, “Le sentinelle dei Variconi”, to protect the entire area from vandalism, to clean trails and dunes, and to survey the species they encounter. Among the main species: *Alcedo atthis, Ardeola ralloides, Ardea cinerea, Ardea purpurea, Asio flammeus, Bubulcus ibis, Circus aeruginosus, Egretta garzetta, Pandion haliaetus, Panurus biarmicus, Phalacrocorax carbo, Phoenicopterus roseus, Platalea leucorodia, Tringa totanus, Tringa nebularia, Upupa epops* (Figure 2).

The dune belt plays a major role in the ecosystem. It is rich in biodiversity but, at the same time, it is victim of a considerable degradation phenomena. Closer to the dune, we found the typical vegetation of this ecosystem type: *Echinophora spinosa, Cakile maritima, Eryngium maritimum, Tamarix, Phragmites communis, Inula viscosa, Crithmum maritimum.* Near the river’s estuary, we found vegetation belonging to *Salicornia* and *Juncus genera*. At the area’s borders, there are small numbers of *Populus, Eucalyptus, Ulmus* and *Phragmites* (Figure 3).

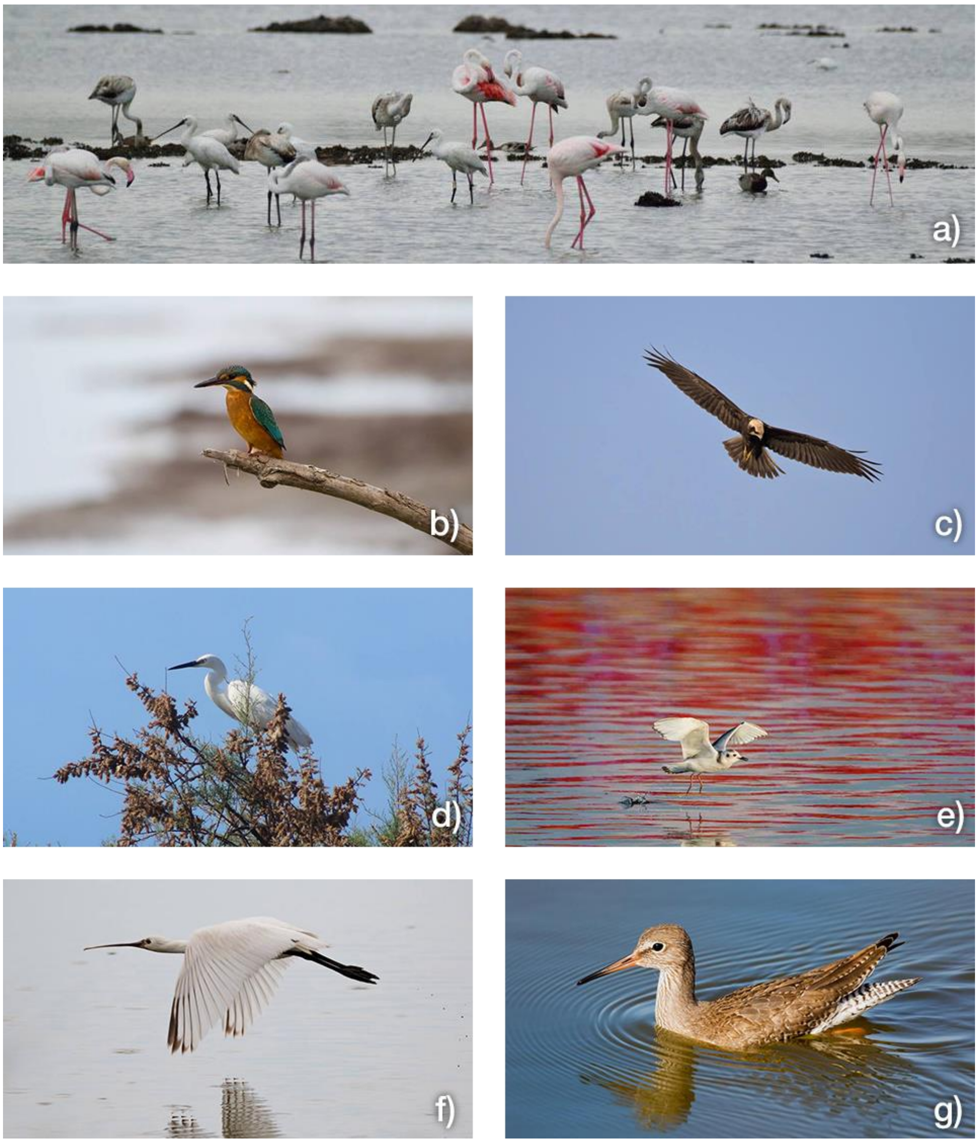

Figure 2: Main *avifauna* species present at Variconi Oasis: a) *Phoenicopterus roseus*; b) *Alcedo atthis*; c) *Circus aeruginosus*; d) *Egretta garzetta*; e) *Larus minutus*; f) *Platalea leucorodia*; g) *Tringa totanus*. Source: Le sentinelle dei Variconi facebook_group. Reprinted with permission.

Figure 3: Variconi Oasis and coastal ecosystem: a) pathway near a pond surrounded by species of *Juncus* genus; b) dune, detail of lowered underwater anti-erosion barrier; c) dune, specimens of *Echinophora spinosa*, *Cakile maritima* and *Eryngium maritimum*, presence of waste and debris.

The characteristics of the socio-economic system and the preliminary information would lead to hypothesize dune soil degradation due to processes such as erosion, compaction, salinization, organic matter decline and waterproofing. Again, to better understand the situation in place, using integrated monitoring actions seems necessary.

2.4 Geo-morphological context

The Volturno basin has an area of 5634 km^2. It is the sixth largest river in Italy, 11th longest (175 km) and has the highest mean annual flow of South Italy: approximately 102 m^3/s (Isidori *et al*.,2004). The river is characterized by minimum flow in summer and overflow in autumn and spring.

The *Domitian* seaboard is mainly formed by low and sandy coasts, stretching for about 50 km from Garigliano's plain up to Monte di Procida. The coastal area is greatly compromised by erosion, due mainly to natural factors such as wind and sea.

Alberico and Pelosi (2016), researchers from IAMC-CNR institute, showed a shore line evolution analysis from the beginning of 1800 to the present days, through the comparative and geo-referenced analysis in GIS (Geographic Information System) of historical maps, regional technical maps, orthophotos and satellite images.

Until the 50s, a general stability of the coastline was recorded.

Afterwards, due to anthropic over-exploitation of the territory, that has led to intense urbanization and industrialization, increasing agricultural and livestock activities and construction of coastal structures, there has been a significant morphological change of the coast and an alteration of the natural landscape.

The result was the loss of thousands of cubic meters of territory, especially in the areas close to the river's mouth. The first significant erosion events occurred in the 60', following the economic boom and the population increase. During these years, the shoreline next to the Volturno's mouth was eroded with average rates of 3.6 m / year. Subsequently, the situation was as follows:

- From 1989 to 1997, near the mouth of the Volturno River, average rates of erosion between -3 and -6 m / year were recorded.
- From 1997 to 2004, the left bank of the Volturno river (our study area) is still eroding, while the right bank undergoes a progress phase.
- From 2004 to 2010, the left bank of the Volturno River starts showing the first signs of progress, while the areas located to the north of the right bank and the far south of the left bank are eroding.

To better understand the different dynamics that affected the right and left side of the Volturno's mouth, it was necessary to conduct researches to obtain valuable information from knowledgeable people. In the 60s there was a violent overflowing of

Volturno river, that damaged both left and right side of the estuary. To limit the damage on the right of the mouth, Bagnara area, a natural lake was converted in a basin to collect the waters of a possible flooding. Unfortunately, not respecting all safety criteria, this intervention proved to be unsuccessful. The lake soon became filled with sand and lost its protective function. The result is that still today the entire coastal strip to the left of the estuary is subject to a very severe erosion and loss of territory. To the left of the mouth, Variconi area, it was decided to build an underwater anti-erosion barrier to protect the natural oasis. Unluckily, the firm in charge of the work, being linked to criminal associations, did not do a good job from a structural point of view; in addition, it was blamed for stealing large quantities of sand. This caused a progressive lowering of the underwater anti-erosion barrier, neutralizing its protective function. In recent years, because of coastal storms, a large quantity of sand settled between the barrier and the coastal strip: as a secondary effect, that allowed a slowing down of the erosion phenomenon (Figure 4) (Bruno D'Alessandro's personal communication).

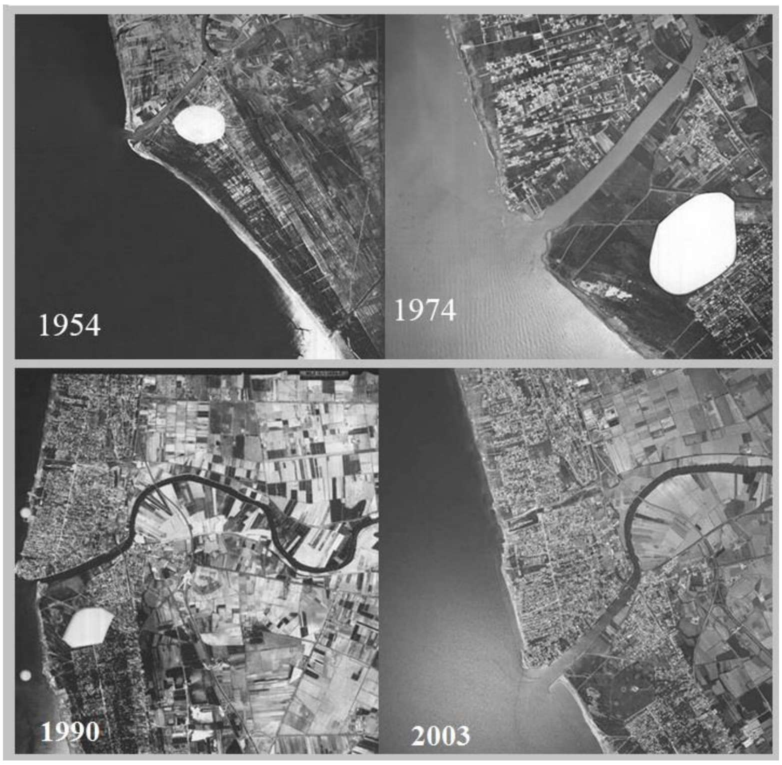

Figure 4: Comparison of shorelines in four different decades, showing soil loss during the first three decades and a slight improvement in the 2000s. Pictures courtesy of http://www.igmi.org.

3 Materials and methods

In order to fully understand the potential presence and the dynamics of the seawater intrusion at Castel Volturno municipality, and given the complexity of the process in question, a multidisciplinary approach was planned. This approach is characterized by the application of stable oxygen (O) and carbon (C) isotope techniques to different water pools (O), soil (O) and plant samples (O and C), coupled with gas exchange measurements, to determine physiological status of the elm (Ulmus minor), our bio-indicator species (Esposito *et al.*, 2014), which is present all over the transect study, along the SP333 roadside. Furthermore, remote sensing was applied to monitor vegetation cover and the Electrical Resistivity Tomography (ERT) geophysical methodology was used to monitor the salinization of groundwater.

3.1 Study area and sampling campaigns

Within the study area (Campania region, South Italy), it was located a transect - approximately four kilometres long - (Figure 5) connecting the mouth of the Volturno river (41°1'23.60" N, 13°55'47.69" E) to the hinterland (41°2'29.87" N, 13°58'35.76" E), crossing three different ecosystems:

- the coastal area with Variconi oasis (Figure 5, points A-B-C-D);
- the urban area (Figure 5, points E, F, G);
- the agricultural area (Figure 5, points H, I, J, K, L, M, N, O).

Figure 5: The study transect from the coast, close to the mouth, to the inner part representing riverine and estuarine areas of Volturno river. The first part of the transect (points A-B-C-D) within the coastal area is located into Variconi oasis. The second part (points E, F, G) is within the urban area while the third one (points H, I, J, K, L, M, N, O) falls in the agricultural area. The water table was sampled from six wells numbered from 1 to 6. Four high resolution Electrical Resistivity Tomography (ERT) profiles were achieved in the rural area in July 2014 (dash symbol). Map courtesy of Google Maps.

After some initial inspections, two experimental campaigns were carried out in October 2013 and in July 2014.

Since the measurement campaigns were carried out during the summer and autumn season, they are characterized by different weather. Specifically, in October 2013 rainfalls in the area amounted to 44.6 mm, while - uncommonly - in July 2014 they amounted to 75.2 mm: that was confirmed to be the rainiest July in the last 10 years (Figure 6). Mean values of monthly minimum and maximum temperatures were, respectively, 9.5°C and 28.3 °C in October 2013 (slightly higher, when compared to the trends of the last decade), and 11.8 °C and 32.6 °C in July 2014, (with slightly lower minimums/higher maximums than the mean monthly values in the last 10 years). All weather data were taken from the National Agro-meteorological database (BDAN, http://cma.entecra.it/Banca_dati_agrometeo).

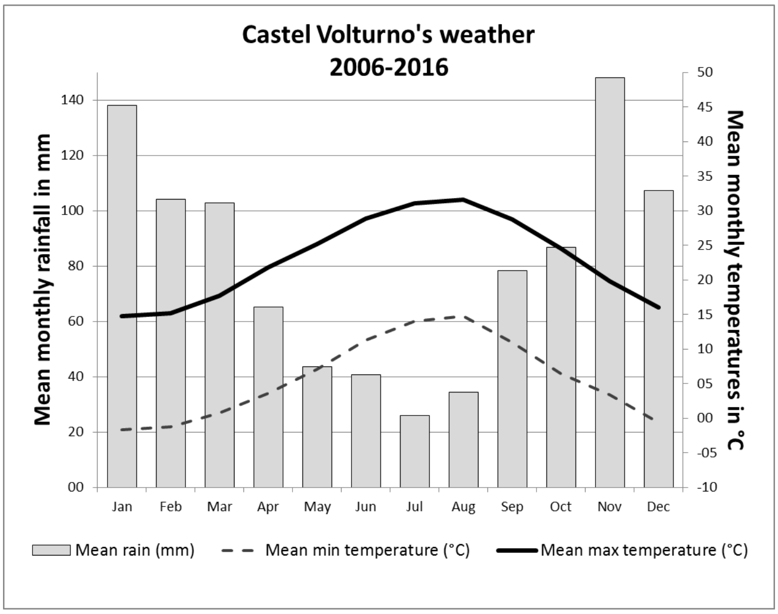

Figure 6: Castel Volturno monthly average rainfall and temperature, with reference to the 2006-2016 decade. Grey bars represent mean monthly rainfall in mm, the continuous black line shows the mean monthly maximum temperatures in °C while the dashed line shows the mean monthly minimum temperatures in °C.

Several samplings were made in the whole transect: leaves and small branches of elm and soil (Figure 5, points A-O; Figure 7), water from wells (Figure 5, points 1-6; Figure 7), and elm leaf gas exchange measurements (Figure 5, points D, G, I, J, K, N, O). Geophysical surveys (Figure 5, dash symbol) were conducted as well.

Figure 7: Leaves-twings (a) and water (b) samplings, and leaf gas exchange measurements (c).

3.2 Stable isotope technique

In the periodic table, some elements have different forms. They are defined isotopes: atoms whose nuclei contain the same number of protons, but a different number of neutrons. These small mass differences cause different physiochemical properties of the molecules. Stable isotopes do not decay into other elements, while unstable isotopes are radioactive and will decay into other elements. There are ~300 stable isotopes in nature and ~1200 unstable (radiogenic). Only 21 elements are "pure" and have just one stable isotope.

In this study, we applied a multiple stable isotope analysis of carbon (C) and oxygen (O), to provide a powerful, not-invasive tool to investigate plant physiological responses to environmental conditions, for tracing biogeochemical processes across spatio-temporal scales and interrelation between water and carbon fluxes (Farquhar *et al.*,1989, Yakir and Sternberg, 2000, Flanagan *et al*., 2005, Augusti and Schleucher 2007).

The natural abundance of C and O isotopes is: ^{12}C = 98.89% (atom contains 6 protons and 6 neutrons) ^{13}C = 1.11% (atom contains 6 protons and 7 neutrons) and ^{16}O = 99.76% (atom contains 8 protons and 8 neutrons) ^{18}O = 0.21% (atom contains 8 protons and 10 neutrons).

Carbon isotope discrimination ($\Delta^{13}C$) analysis in plant material has been proved a good indicator of ecological change (Dawson and Siegwolf, 2007) and ecophysiological traits of adaptive significance (Brendel *et al*.,2003; Lauteri *et al*.,2004; Scartazza *et al*.,2014) to abiotic constraints like salinity (Brugnoli and Lauteri, 1991; Monteverdi *et al*.,2008).

Nier & Gulbransen (1939) were the first scientists to associate the C isotopic differences due to diffusional and kinetic fractionation processes during CO_2 fixation in leaves (Farquhar & Richards, 1984) to trace plant ecological processes. Leaf isotopic discrimination depends on several environmental conditions that can affect stomatal conductance (CO_2 supply) and/or photosynthesis (CO_2 demand), that provide carbon isotopic fractionation. The ^{13}C leaves signal is reflected in all plant compartments and in root and soil in very short timescales (Ekblad and Högberg 2001; Bowling *et al*., 2002). This allows using stable isotopes technique to study carbon allocation under different environmental conditions (Palta & Gregory 1997).

This technique allows us to investigate on a long-term indicator for integrated water use efficiency (WUE) of plants in the growing process (Taylor *et al*.,2013, Medrano *et al*., 2015), subsisting, for the C3 plants, a linear and negative relationship between $\Delta^{13}C$ and WUE. This parameter is the ratio of carbon gain to water losses (Brugnoli and Farquhar, 2000) and generally increases under drought and soil salinization, that is when soil and plant water potentials decrease (Ehleringher and Dowson, 1992).

In order to investigate the water movements along the soil-plant-atmosphere continuum and to monitor the water source nature, the oxygen isotope composition ($\delta^{18}O$) of different samples (branches, water well and soil water) was analysed (Dawson *et al.*, 2002; Lauteri *et al.*,2006; Barbour, 2007). Actually, owing to Rayleigh fractionation phenomena during clouds formation and condensation, continental water is usually characterized by negative values of $\delta^{18}O$ (Craig, 1961; Dansgaard, 1964). On the contrary, $\delta^{18}O$ values in sea water are typically close to 0‰, approaching the reference value of the VSMOW. Particularly important is the isotopic analysis involving the branches, since in absence of isotopic fractionations during the water uptake from the roots to the twigs (White *et al.*,1985; Ehleringher and Dowson, 1992), the $\delta^{18}O$ of xylem water reflects the one from source water. This type of investigation is extremely useful in presence of compromised water sources.

3.2.1 Isotopic determinations

The first step was the determination of leaf dry matter and leaf soluble sugar carbon isotope composition ($\delta^{13}C$) and carbon isotope discrimination ($\Delta^{13}C$). Fully expanded leaves, subject to different solar exposure, were sampled from all around each tree crown. Then, they were frozen with liquid nitrogen, dried and reduced to a fine powder with the assistance of a mortar in the laboratory. From the obtained powder, a little part (about 0.5 mg) was taken to analyze $\delta^{13}C$ on bulk leaf dry matter; another sub-sample (about 100 mg) to extract the soluble sugar, extracted in water and purified by means of anionic (DOWEX 1x2 Cl^- form, strongly basic) and cationic (DOWEX 50Wx4 hydrogen form) exchange resins (Lauteri *et al.*, 2014). The obtained mixtures were freeze-dried and a sub-sample of about 0.5 mg were used for $\delta^{13}C$ on leaf soluble sugars.

Organic samples were combusted into an elemental analyzer (NA 1500; Carlo Erba) and the carbon dioxide formed after combustion was then transported to a mass spectrometer (Isoprime Ltd, Isoprime) through a helium stream, as described in Alessio *et* *al*., (2004). In order to calculate $\delta^{13}C$, isotope ratios were measured as the abundance of the minor, heavier isotope of the element to the major, lighter isotope:

$$R_{sample}={}^{13}C/{}^{12}C \quad (1)$$

Than $\delta^{13}C$ was calculated according to the Vienna Pee Dee Belemnite (VPDB) standard:

$$\delta^{13}C = R_{sample} / R_{VPDBstandard} - 1 \quad (2)$$

For carbon isotopes, it is preferred to refer to isotopic discrimination, as explained by Farquhar *et al*. (1989):

$$\Delta^{13}C = (\delta_{air} - \delta_{plant}) / (\delta_{plant} +1) \quad (3)$$

where δ_{plant} is $\delta^{13}C$ of the leaf bulk matter and δ_{air} is assumed to be -9,0‰.

The second step was the determination of oxygen isotope composition of xylem water extracted from twigs, water extracted from soil and groundwater taken from the wells. In order to extract water from the branches and the soil, a cryogenic vacuum line was needed, as described in Alessio *et al*., (2004). A sub-sample of water samples (about 100 μl) were analyzed by an isotope ratio mass spectrometer (Isoprime, Isoprime Ltd, DE) coupled with a multiflow prep-system (BIO, Isoprime Ltd, DE) and an autosampler (222XL, Gilson, Inc. Middleton, WI, USA) in order to determine $\delta^{18}O$.

Even in this case, the first step was the determination of the isotopic ratio between ^{18}O and ^{16}O:

$$R_{sample}={}^{18}O/{}^{16}O \quad (4)$$

$\delta^{18}O$ was calculated according to the international Vienna Standard Mean Ocean Water (VSMOW) as:

$$\delta^{18}O = R_{sample} / R_{VSMOWstandard} - 1 \quad (5)$$

To get a more complete view on the water nature, in 2014 pH and electrical conductivity (EC) were measured for each water wells sample. EC is reported at the reference temperature of 25 °C.

In order to have reliable data, each measure was performed on three sample replicates. It should be noted that, given the homogeneity of the wells' water samples, they show a standard error lower than the instrument precision; because of that, it is always approximated to 0.0 in ‰ notation.

3.3 Leaf gas exchange

Elm leaf gas exchange measurements of CO_2 and H_2O were performed in situ on 28th and 29th July 2014, approximately between 9 am and 5 pm, solar time, by using a portable gas exchange system (LI-COR 6400, LI-COR Biosciences Inc., Lincoln, NE, USA) on elms trees, chosen along the different parts of the transect (Figure 3).

For each sampling point along the transect, at least three small branches with different exposure were cut after submersion into a water pot, in order to avoid xylem cavitation. From each branch, three young, well expanded and exposed leaves were sequentially clamped in the gas-exchange cuvette equipped with a modulated light fluorimeter (LI-COR 6400-40). Net photosynthesis (*A*), transpiration (*E*), stomatal conductance (g_s) and internal CO_2 concentration (C_i) were derived from the ancillary gas exchange measurements, according to the model described by von Caemmerer and Farquhar (1981). Simultaneously to gas exchange parameters, chlorophyll and fluorescence were recorded as well. Within the cuvette, the leaves were exposed to saturating photosynthetic photon flux density (PAR; 1800 μmol m^{-2} s^{-1}), at CO_2 ^{c}oncentration of 400 ppm and air temperature of 26 $^{\circ}$C, relative humidity ranging between 36% and 50%. Intrinsic WUE (also defined as WUE_i - instantaneous WUE) was calculated as the ratio between *A* and *gs*. WUEi increases either due to decrease in *gs* or an increase in A, intercellular CO2 declines and discrimination decreases (inverse relationship) (Cregg and Zangh 2000).

3.4 Remote sensing - NDVI

Landsat orthorectified images (path 189-190, row 031-032) were acquired through the USGS EarthExplorer portal (http://earthexplorer.usgs.gov/); six data acquired by the sensors Landsat-8 ETM+ (Enhanced Thematic Mapper) and two data by Landsat-5 TM (Thematic Mapper), respectively, were used in the present study. Landsat images with 30 m spatial resolution in Near Infra-Red (NIR) and Red (R) band were considered and the NDVI was calculated as:

$$NDVI=(NIR-R)/(NIR+R) \quad (6)$$

It is well-established that this index varies between -1 and 1 and is well correlated with vegetation status (Huete *et al*., 2002). The radiometric calibration of the Landsat data was carried out to convert the digital numbers recorded from the sensors to reflectance values. To evaluate the vegetation cover in an area of interest of about 40 km^2 (AOI), the mean value (m) and the standard deviation (σ) of seasonal NDVI for 2013 (from 02/06/2013 to 29/07/2013) were calculated. These values were considered as the seasonal vegetation focus of AOI. The temporal evolution of vegetation cover was performed comparing the m values of 2013 >0,2 vs 1984 and 2003 (for date 18/06/1984 and 23/06/2003). A gain in vegetation cover, can be found, if m > NDVI1984/2003+3σ, a stable vegetation if NDVI1984/2003-3σ ≤ m ≤ NDVI1984/2003+3 σ and a loss in vegetation cover if m < NDVI1984/2003-3σ.

3.5 Geophysical methods

Since water resistivity ranges from 0.2 to over 1000 Ω·m, and depending on its ionic concentration and TDS content, it can be considered related with groundwater salinity. Sodde and Barrocu (2006), Van Dam and Meulenkamp (1967) classified the resistivity values as follows: 40, 12 and 3 Ω·m for fresh, brackish and saline water, respectively. Zohdy *et al*. (1993) proposed a classification based on resistivity values and lithology (Table 1).

Different electrical geophysical methods can be applied to detect the saltwater intrusion, such as airborne electromagnetic (Viezzoli *et al*., 2010), Vertical Electrical Sounding (VES) (Massoud *et al*., 2015), and borehole electrical conductivity (EC) measurements (Kim *et al*., 2006). However, the best results are generally obtained using electrical resistivity tomography (ERT) (Gemail *et al*., 2004; Chitea *et al*., 2011; Gurunhada Rao *et al*., 2011; Zarroca *et al*., 2011) which, pursuing the determination of the apparent resistivity (ρ) of the soil along the transect, allows to discriminate salt and freshwater.

Table 1: Resistivity of water and sediments, proposed by Zohdy *et al*., 1993.

Resistivity (Ωm)	Sediments	Interpretation
0.5-2.0	Very porous sand, or satured clay	Seawater; very saline water; TDS: about 20000 mg/l
2.0-4.5	Porous sand, or satured clay	Saline water; TDS: about 10000 mg/l
4.5-10.0	Sandy saturated, or sandy clay	Salty brackish water; TDS: about 10000-1500 mg/l
10.00-15.00	Sandy clay, sandy gravel	Brackish water; TDS: about 5000-1500 mg/l
15.00-30.00	Sand, gravel, some clay	Poor quality fresh water; TDS: 1500-700 mg/l
30.00-70.00	Sand, gravel, minor clay	Intermediate quality fresh water; TDS: 100 mg/l
70.00-100.00	Sand, gravel, no clay	Good quality fresh water; TDS: small
More than 100.00	Coarse sand, grave, no clay	Very good quality fresh water; TDS: very small

3.5.1 Geophysical measurements

2D ERT profiles were acquired to determine the presence of saline intrusion through the apparent resistivity (ρ) of the soil. The acquisition was obtained merging measurements of electric current intensity and voltage through separate couples of electrodes, immersed in the ground surface and connected to a georesistivimeter through multichannel cables, adopting the Wenner-Schlumberger array configuration (Pazdirek and Blaha, 1996). This configuration is particularly recommended in areas characterized by both lateral and vertical variations of resistivity (Loke and Barker, 1996).

As a preliminary study to verify a possible saltwater intrusion phenomenon in the area, three low resolution and large scale ERT profiles were executed in coastal, urban and agricultural areas, respectively.

The first profile was acquired in January 2013 near Variconi oasis, within the coastal area. This profile was carried out using 48 electrodes with a unit spacing of 4 m, the total length being 188 m. The second profile was acquired in the urban area, in February 2013, for a length of 475 m (96 electrodes, 5 m spacing). Lastly, the third profile was obtained in the rural area, in February 2013, with a total length of 950 m (96 electrodes, 10 m spacing).

The following year, July 2014, in order to get more information on the most productive area, four high resolution near surface ERT profiles were acquired in the rural area. In this case, the acquisition was obtained using 24 electrodes, with electrode spacing of 50 cm. Once acquired, data have been inverted to obtain the two-dimensional sections of resistivity.

For all profiles, data inversion started from a discretized model of the investigated area, built starting from average apparent resistivities on measured pseudosections. The inversion procedure was carried out by RES2DINV program, version 6.1, (GEOTOMO SOFTWARE, 2002) and uses a smoothness-constrained least-squares routine (deGroot-Hedlin and Constable, 1990), implemented into Occam's optimization algorithm (LaBrecque *et al*., 1996).

3.6 Statistical analyses

One-way analysis of variance (ANOVA) was applied in order to evaluate the differences among mean values. Shapiro-Wilk test of normality and separation of means was performed by Student-Newman-Keuls test.

Linear regressions were used to analyze the relationships between Δ values of leaf dry matter and leaf soluble sugars, between $\delta^{18}O$ of xylem water, gas exchange and fluorescence measurements and between leaf Δ, $\delta^{18}O$ of xylem water and Φ_{PSII}.

Statistical analysis was conducted using SigmaPlot 12.0 (Systat Software, USA).

4 Results

4.1 Isotopic analyses

At isotopic level, the first noticeable fact is a wide $\Delta^{13}C$ variability for leaf dry matter (Δ_{dm}) sampled on *U. minor*, for both campaigns. In fact, Δ_{dm} ranged from 17.7‰ to 21.6‰ in October 2013 and from 18.4‰ to 20.8‰ in July 2014 (Table 2; Figure 5).

Table 2: Carbon isotope discrimination of both leaf dry matter (Δ_{dm}) and leaf soluble sugars (Δ_{ss}) and oxygen isotope composition ($\delta^{18}O$) in xylem water determined along sixteen points (from A to O) along the study transect during October 2013 and July 2014 sampling campaigns. The first part of the transect (points A-B-C-D) is within the coastal area (Cs), the second part (points E, F, G) is within the urban area (Ru) while the third part (points H, I, J, K, L, M, N, O) falls in the rural area (Ru). The average values ± standard error (SE) of the whole transect and of the three different areas for 2013 and 2014 sampling campaigns are shown (n.d., not determined). ** or * on the average values indicate, respectively, highly significant or significant difference of the specific mean value, in respect to the other means in column.

Transect Point	**Area**	**Leaf Δ_{dm} (‰)**		**Leaf Δ_{ss} (‰)**		**Xylem water $\delta^{18}O$(‰)**	
		October 2013	July 2014	October 2013	July 2014	October 2013	July 2014
A	Cs	18.7	19.4	17.5	17.6	-5.2	-4.7
B	Cs	19.3	20.8	20.0	18.2	-4.5	-4.8
C	Cs	19.6	n.d.	19.6	n.d.	-4.6	n.d.
D	Cs	17.7	18.5	17.5	16.2	-4.3	-6.1
E	Ur	21.6	20.6	22.9	18.8	-5.8	-6.1

F	Ur	19.8	19.4	19.5	18.7	-6.1	-5.7
G	Ur	19.8	19.8	18.6	18.8	-4.6	-5.8
H	Ru	19.9	18.9	21.0	17.7	-5.5	-5.2
I	Ru	20.1	19.8	18.8	17.8	-4.5	-5.2
J	Ru	20.6	19.3	20.6	18.3	-3.8	-5.8
K	Ru	19.9	18.4	20.1	16.5	-3.8	-5.7
L	Ru	19.3	n.d.	18.2	n.d.	-5.2	n.d.
M	Ru	21.5	n.d.	22.4	n.d.	-5.4	n.d.
N	Ru	20.2	9.5	19.8	18.3	-4.7	-5.40
O	Ru	19.7	20.4	20.6	19.6	-4.3	-5.40
Mean value ± SE		19.9 ± 0.3	19.6 ± 0.2	19.8 ± 0.4	18.0 ± 0.2	-4.8 ± 0.2	-5.5 ± 0.1
Coastal Area ± SE		18.9** ± 0.4	19.6 ±0.4	18.7 ± 0.7	17.3* ± 0.4	-4.7 ± 0.2	-5.2 ± 0.5
Urban Area ± SE		20.4 ± 0.6	19.9 ± 0.2	20.3 ± 1.3	18.8* ± 0.3	-5.5 ± 0.5	-5.9* ± 0.1
Rural Area ± SE		20.2 ± 0.2	19.4 ± 0.2	20.2 ± 0.5	18.0 ± 0.3	-4.7 ± 0.2	-5.4 ± 0.1

Within the three study sub-areas, different trends are shown. In October 2013, the Δ_{dm} average values are basically lower within coastal area (~ 18.9‰; Table 2), while in the other two areas slightly higher values (~ 20.4‰ and ~ 20.2‰; Table 2) are recorded; the same trend is observed in the following sampling campaign (July 2014). Focusing on leaf soluble sugars, in October 2013 the average $\Delta^{13}C$ (Δ_{ss}) is almost similar to the corresponding leaf dry matter values and constant for all three sub-areas. In July 2014, however, it is more diversified and remarkably lower than the corresponding value in leaf dry matter. This occurs because Δ_{ss} depends on seasonality and in particular on temperatures. That is highlighted plotting Δ_{dm} and Δ_{ss} for both campaigns (Figure **8**), where a higher span in October is clearly visible, compared to July.

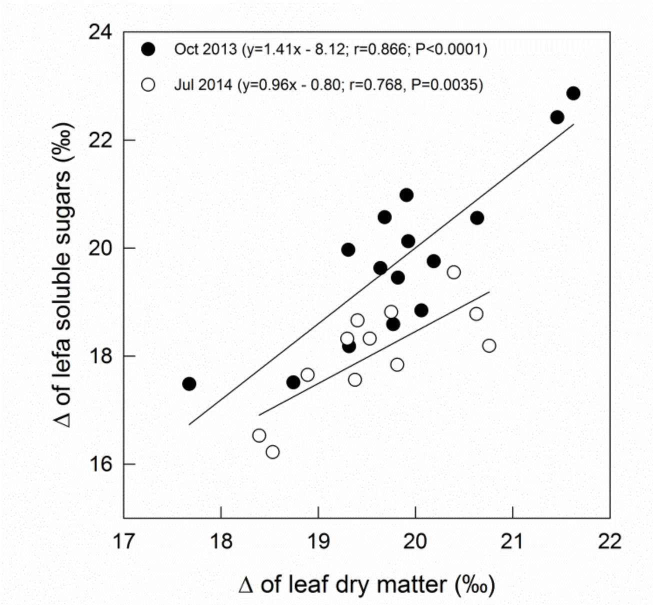

Figure 8: Relationships between Δ_{dm} and Δ_{ss} during October 2013 and July 2014. Each point represents a different site located along the study transect from the mouth of the Volturno river to the inner agricultural area. Each value represents the average of three replicates. The values of the correlation coefficient (r) and the significance level (P) of the linear regressions are shown.

Regarding $\delta^{18}O$ of xylem water, values ranged from -3.8‰ to -6.1‰ in October 2013 and from -4.7‰ to -6.1‰ in July 2014 (Table 2), with average values within the urban area lower than those of the coastal and rural areas. The highest differences, although not statistically significant, occur in October 2013 (Table 2). These values reveal the absence of a clear salinization trend for both campaigns. Also $\delta^{18}O$ of water wells, collected during the same sampling campaigns, shown similar values: from -4.2‰ to -6.1‰ in October 2013 and from -4.4‰ to -6.3‰ in July 2014 (Table 3). Isotopically speaking, these values do not show the presence of sea water (whose reference value is close to 0). However, for the samples taken in July 2014, electrical conductivity measurements (EC) were carried out as well, leading to interesting results. Some water samples have higher EC values, attributable to a significant presence of dissolved salts (Table 3). The most worrying levels are found in the rural area (wells #5, #6 and #7), while moderate values are found in the urban zone (well #2); lastly, lower values with a negligible salt water risk are found near the coast (well #1).

These data are also matching at isotopic level. In fact, in July 2014, well number 6, shows the highest value of EC and $\delta^{18}O$; furthermore, water extracted from soil sampled near the same well (point I, in the rural area), shows the most enriched values of $\delta^{18}O$ (~ -4.2‰, irrespective of depth).

In order to provide an isotopic picture of the soil water available to the plants, a series of soil profiles were sampled during July 2014 (Table 4). The profiles, three steps each (40 – 60 and 80 cm), did not reveal any clear trend in oxygen isotopic composition, either proceeding from the coast to the inner or proceeding in depth. Actually, often the values are discordant. While point I showed higher levels of the heavier isotope, the most depleted $\delta^{18}O$ value was equal to -6.8‰ at point K, 40 cm deep (Table 4).

Table 3: Oxygen isotope composition ($\delta^{18}O$) of water from wells, sampled in October 2013 and July 2014. Each value represents the average of three replicates ± SE (n.d., not determined). The values of pH and electrical conductivity (EC) were measured only on pooled water samples collected on July 2014, at the standard condition of 25°C. For the July sampling data is reported also the $\delta^{18}O$, pH and EC values of the tap water.

Well code	**Area**	**$\delta^{18}O$ (‰)**		**pH**	**EC ($\mu S\ cm^{-1}$)**
		October 2013	July 2014	July 2014	
1	Cs	-4.2 ± 0.0	-5.3 ± 0.0	6.9	406.9
2	Ur	n.d.	-6.3 ± 0.0	7.2	940.1
3	Ru	-5.4 ± 0.0	-5.7 ± 0.0	7.1	726.6
4	Ru	-6.1 ± 0.0	n.d.	n.d.	n.d.
5	Ru	-5.3 ± 0.0	-5.9 ± 0.0	7.2	1271.3
6	Ru	-4.6 ± 0.0	-4.4 ± 0.0	7.0	2807.4
7	Ru	n.d.	-5.7 ± 0.0	7.1	1098.9
Mean value		-5.1	-5.6	7.1	1208.5
± SE		± 0.3	± 0.3	± 0.2	±302.5
Tap water		n.d.	-6.7 ± 0.0	7.6	218.5

Table 4: Oxygen isotope composition ($\delta^{18}O$) of water extracted from soil samples taken at three depths (40, 60 and 80 cm) in different sites along the study transect, during the July sampling campaign. Each value represents the average of three soil samples for each depth. The average values ± standard error (SE) for different depth are shown (n.d., not determined).

Transect point	**Area**	**$\delta^{18}O$ of soil water (‰)**		
		40 cm	60 cm	80 cm
D	Cs	-5.6 ± 0.0	-5.9 ± 0.3	-5.5 ± 0.0
G	Ur	-5.3 ± 0.3	-5.1 ± 0.2	-5.7 ± 0.1
I	Ru	-4.1 ± 0.2	-4.3 ± 1.0	n.d.
J	Ru	-5.4 ± 0.2	-6.3	-6.6 ± 0.3
K	Ru	-6.8 ± 0.1	-5.1 ± 0.6	-6.2 ± 0.4
N	Ru	-5.5 ± 0.8	-6.1 ± 0.7	-5.8 ± 0.7
O	Ru	-6.3 ± 0.4	-6.0 ± 0.2	-6.1 ± 0.2
Mean value		-5.5 ± 0.3	-5.5 ± 0.3	-6.0 ± 0.2

4.2 Leaf gas exchange

Leaf gas exchange data showed a high variability. Greater photosynthetic activity (*A*) is found in coastal (point D) and urban (point G) areas, with values of 18 and 21 µmol m^{-2} s^{-1}, respectively. That level tends to decrease dramatically for plants in the rural area, reaching the lowest value of 8.3 µmol m^{-2} s^{-1} (point O) (Figure 6). An improvement of the study on the variables involved in gas exchange can be seen, as in some cases the decreasing of *A* matches with the decreasing of stomatal conductances (g_s) (point O), highlighting stomatal limitations. In other cases, however, relatively low *A* values matched with high values of intercellular CO_2 concentration (C_i), pointing out non-stomatal limitations (points K and N). Furthermore, Figure 9 shows a strong correspondence between g_s and transpiration rates (*E*), and also shows significant variations of the photosystem II along the transect (PSII) operating efficiency (Φ_{PSII}). It is interesting to note that, generally, the quantum efficiency of PSII electron transport in the light matches with photosynthesis rate, but at point K a low *A* value is associated to a high value of Φ_{PSII} (Figure 9), confirming that the photosynthetic activity is impaired by different factors. Among those factors, there could be water's quality and nature: to verify it, in Figure 10 were shown the relationships linking *A*, g_s and Φ_{PSII} to $\delta^{18}O$ in xylem water. As is clearly shown, there are three negative, significant and linear relationships, indicating that enriched water affects photosynthesis, stomatal aperture and electron transport efficiency. With further investigation on the relationships between isotopic and gas exchange data, we note that the parameter Φ_{PSII} was also negatively related to Δ_{dm} (Figure 11, upper panel), with a trend becoming significant in rural area only: that reveals how the efficiency of PSII electron transport in the light decreases when Δ_{dm} increases. Lastly, a positive (although not significant) trend ($P<0.08$) has been observed between $\delta^{18}O$ in xylem water and $\Delta^{13}C$ in leaf dry matter (Figure 11, lower panel).

To get a more complete picture on monitored plants' WUE, the intrinsic/instantaneous WUE (WUEi) was calculated. Then, this parameter was compared with the long-term WUE indicated by isotopic analysis (Figure 12). The two parameters show similar trends, but with different situations. Generally, the WUEi is strongly influenced by field and environmental conditions, so represents the daily status of the plant quite precisely, it is necessary to refer to the long-term WUE in order to gather complete and reliable data.

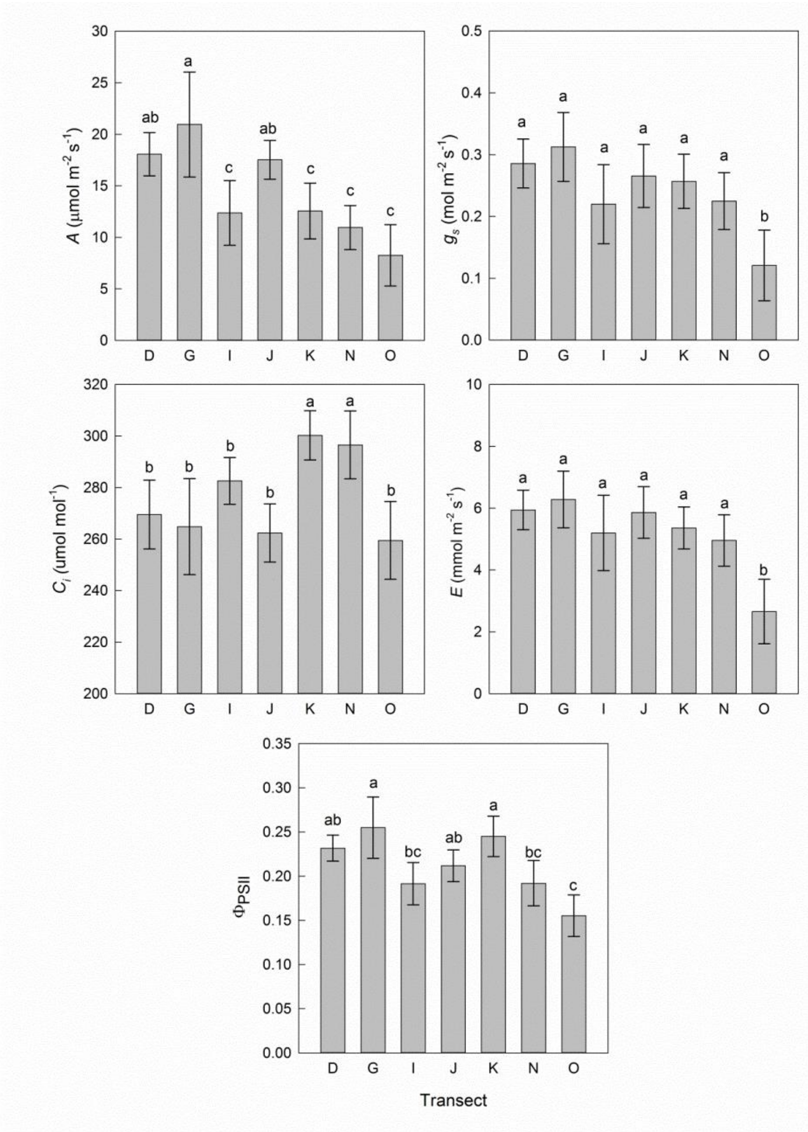

Figure 9: Net CO_2 assimilation rate (*A*) and stomatal conductance (g_s) (upper panel), internal CO_2 concentration (C_i) and transpiration rate (*E*) (middle panel) and actual photochemical efficiency of photosystem II (Φ_{PSII}) (lower panel) determined during July 2014 in different point of the study transect (see Figure 3). Values represent the average of three replicates ± standard error (SE). Data were analyzed independently by one-way ANOVA. For each parameter, data followed by different letters are significantly different ($P \leq 0.05$, Student–Newman–Keuls).

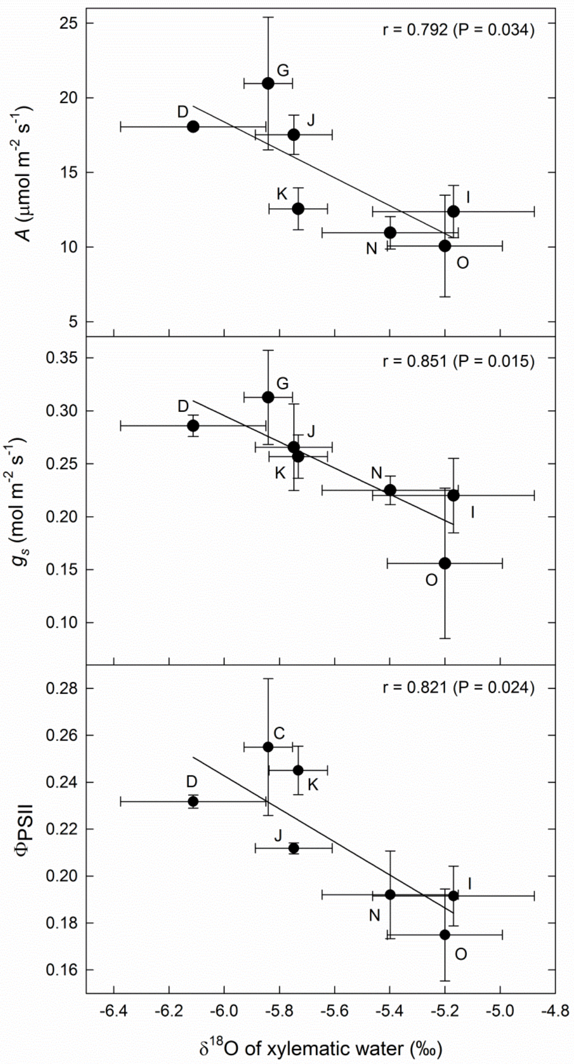

Figure 10: Relationships of oxygen isotope composition ($\delta^{18}O$) of xylem water with CO_2 assimilation rate (*A*, upper panel), stomatal conductance (g_s, middle panel) and actual photochemical efficiency of photosystem II (Φ_{PSII}, lower panel). Each value represents the average of three replicates ± standard error (SE). The letters indicate different points along the study transect (see Figure 3). The values of the correlation coefficient (r) and the significance level (P) of the linear regressions are shown.

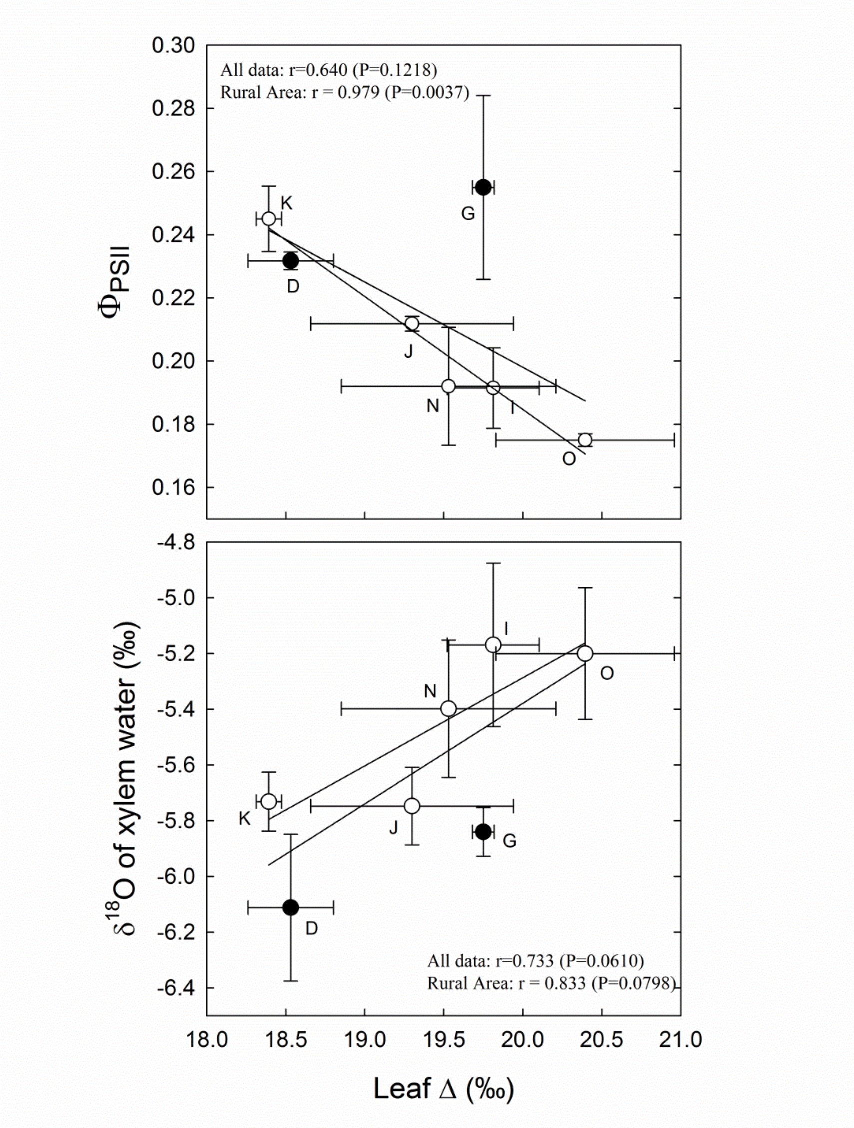

Figure 11: Relationships between carbon isotope discrimination of leaf dry matter (Δ_{dm}), actual photochemical efficiency of photosystem II (Φ_{PSII}; upper panel) and oxygen isotope composition ($\delta^{18}O$) in xylem water (lower panel). Each value represents the average of three replicates ± standard error (SE). The letters indicate different points along the study transect (see Figure 3). The values of the correlation coefficient (r) and the significance level (P) of the linear regressions are shown for both for all data and only for the rural area.

Figure 12: Variation of intrinsic WUEi (A/gs - continuous black line) and Δ_{DM} ^{13}C (inversely related to long term WUE - dashed grey line) along the transect.

4.3 NDVI compares

During the whole research, we used the NDVI index in to determine whether in the present (2013) there has been an increase in vegetation compared to the previous decade and thirty years (2003 and 1984.) The results reported in Table 5 show a net gain in vegetation cover for both the years in comparison.

Table 5: NDVI estimation of gain and loss in vegetation for year 2013 compared to years 1984 and 2003.

NDVI based	**Gain**		**Loss**	
comparison	[%] of AOI	Km^2	[%] of AOI	Km^2
vs. 1984	12.4	4.5	5.9	2.1
vs. 2003	18.5	6.7	1.6	0.6

Of course, when using this analysis method, it is not possible to discriminate the type of vegetation detected; however, as is clearly visible through satellite pictures, the situation has improved in recent years, from a quantitative point of view (Figure 13). This was possible thanks to the dedication of “Le sentinelle dei Variconi” association's volunteers in attempting to protect the vegetation and the whole ecosystem through beach and trails cleaning campaigns, creation of ecological paths, installation of information panels and huts for bird-watching.

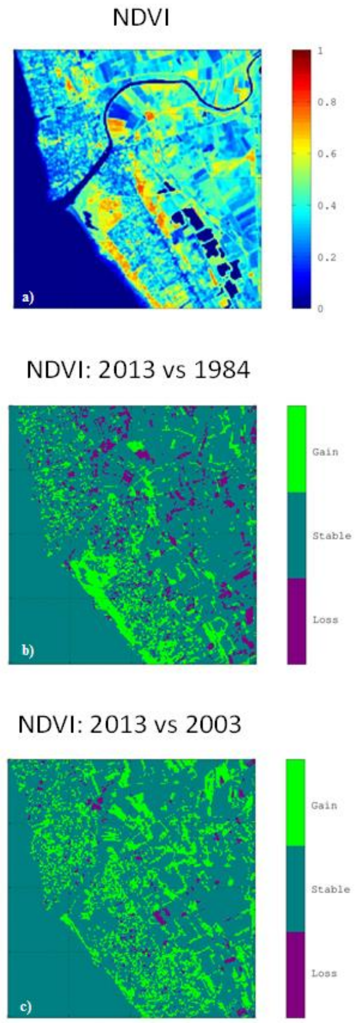

Figure 13: Panel a) shows a NDVI picture, taken in 2013. The values are referred to: a dense vegetation canopy (from 0,3 to 0,8), soil (from 0,1 to 0,2) and clear water (barely positive or even slightly negative values). The panels b) and c) show a comparison of vegetation cover between 2013 and 1984 - 2003, respectively.

4.4 ERT profiles

The low-resolution ERT profiles in the three investigated areas provided general information on a possible saline intrusion (Figure 14). The coastal ERT, acquired at the mouth of the river near the Variconi protected area, shows low resistivity values (0,4 Ωm to 1 Ωm) as early as 2 meters from ground level; that indicates the presence of a salt water level. This profile was measured on a land located on the side of the river mouth, and near the coast. In this case, the intrusion of sea water is obvious and evident. The profiles gathered in urban and rural areas, in the first 5 m of depth, showed a first resistive layer characterized by the absence of salt or brackish water. A verification during the measurement campaign has proved that the saltwater strip highlighted in the urban area was an error. In that point, most likely, there are underground pipes that may have disturbed the measurements. Lastly, in urban and rural areas profiles, at a depth of about 13-15 m - in correspondence of low resistivity values (1 -5 Ωm) - a salt and brackish groundwater layer with a high concentration of salts was observed.

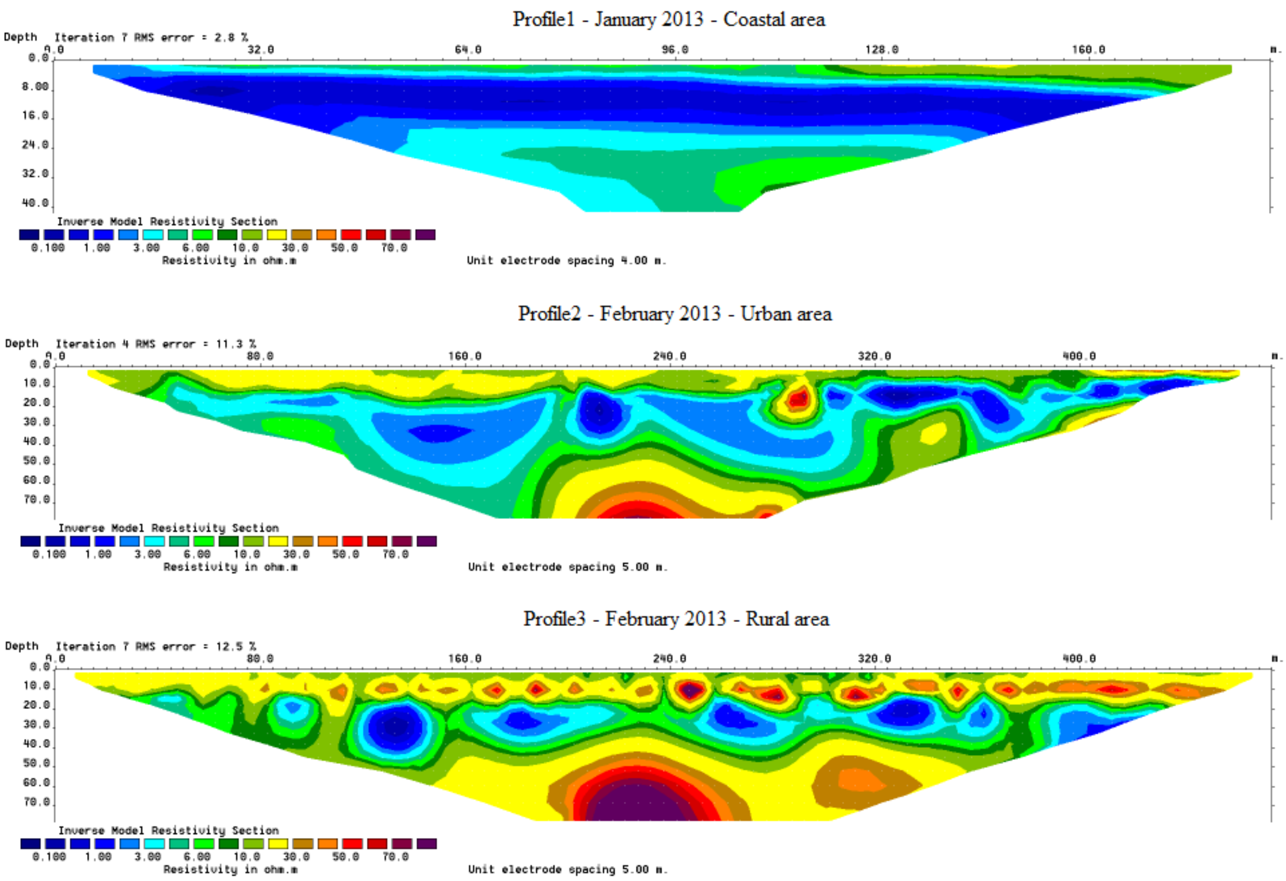

Figure 14: Low resolution Electrical Resistivity Tomography (ERT) profiles carried out during 2013 at coastal (Profile1), urban (Profile 2) and agricultural areas (Profile3).

In order to obtain more accurate investigation in the most economically interesting area, four high resolution ERT profiles were made on the rural part, where there is a high water pumping demand for agricultural purposes. These profiles were acquired with a smaller distance between the electrodes, so that the resolution of the superficial layers is higher. The four profiles (Figure 15) shown medium or low values of resistivity, suggesting the presence of freshwater. Table 6 shows the minimum values of resistivity in Ωm for each ERT profile.

Table 6. Minimum resistivity values determined by the Electrical Resistivity Tomography (ERT) sections carried out on July 2014 in different sites of the rural area along the study transect. (see Figure 1 and supplementary material).

ERT_ Sections	**Minimum resistivity values (Ωm)**
Section _1 (point I)	3.71
Section _2 (point K)	7.54
Section _3 (point M)	7.11
Section _4 (points N-O)	6.66

In ERT section #1 (at point I) the groundwater level was about 1m deep, with resistivity values ranging between 3.7 Ωm and 10 Ωm. Also the ERT section #2 (at point K) indicated a shallow water table (about 0.5 m deep), although characterized by higher resistivity values (7.5-15 Ωm) as compared to the other profiles. This level was sub-outcropping in the ERT section #3 (at point M), where the values of resistivity reaches a minimum around 7 Ωm. Finally, in ERT section #4 (between points N-O) the groundwater level was present at a depth of 1 m with resistivity values between 6.6 Ωm and about 10 Ωm. As a general result, all the high-resolution sections show low resistivity values (3-15 Ωm), which probably indicate the presence of aquifer with moderate content of dissolved salts (Chitea *et al*., 2011). This means that, at a depth explored by the roots, saline – salty/brackish – brackish water was found (Zohdy *et al*., 1993).

This information was not detectable in the low-resolution profile corresponding to the rural area, since the larger distance between the electrodes allows to get profiles at high depth, but with low surface resolution.

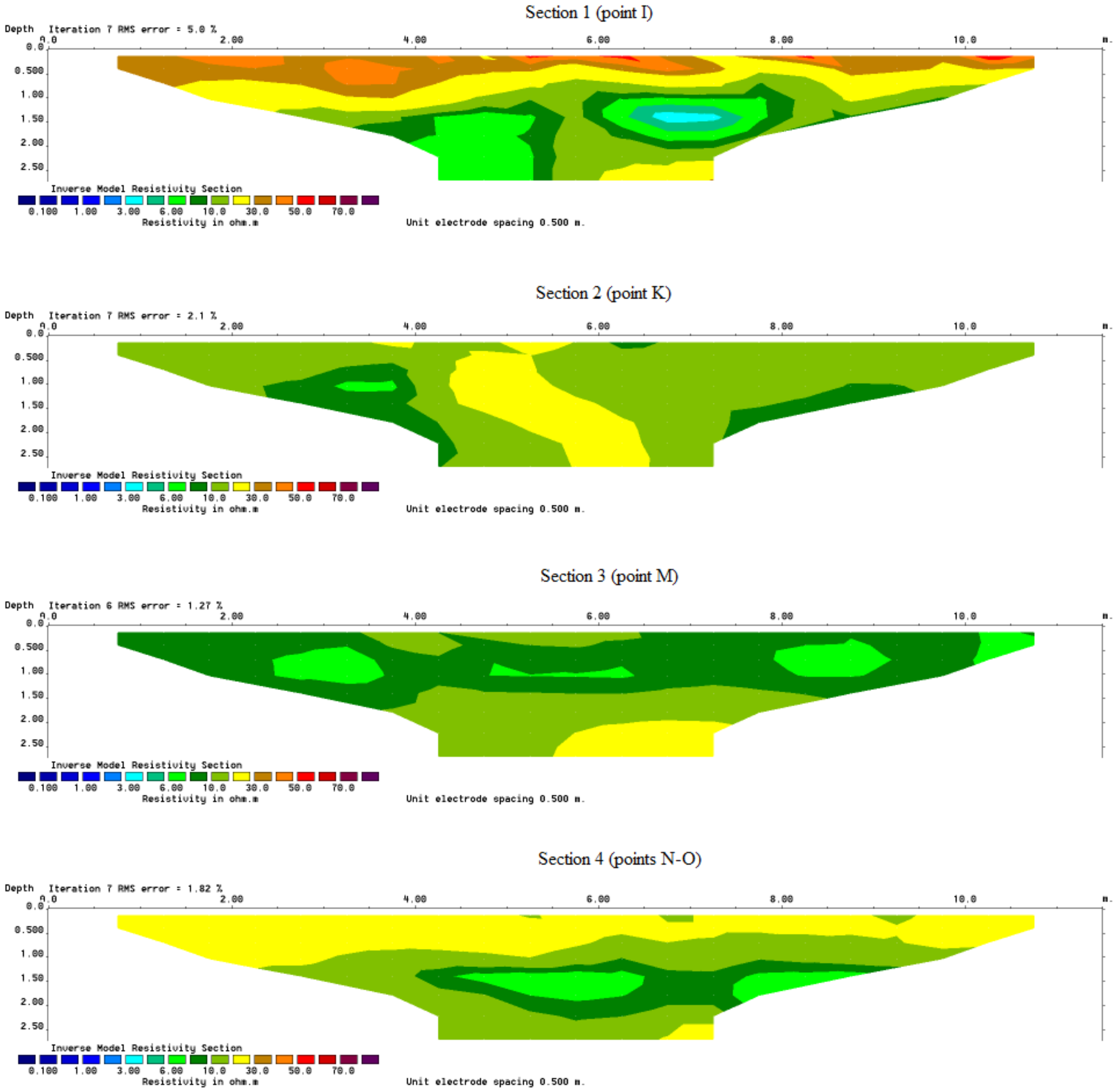

Figure 15: High resolution Electrical Resistivity Tomography (ERT) profiles carried out on July 2014 in different points of the rural area along the study transect (see Figure 3).

5 Discussion

Secondary salinization is a growing issue in the whole world and, particularly, in the Mediterranean regions, characterized by prolonged drought summers and high water demand from population along the coastal areas (Postiglione, 2002; Monteverdi *et al.*, 2008), where excessive pumping of water affects the fresh-saltwater interface causing a phenomenon known as saltwater up-coning (Aharmouch and Larabi, 2001), which consists in the movement of saltwater from the bottom upwards. Combined with groundwater / sea level variations and coastal erosion, this process induces a potential "seawater intrusion"

At Castel Volturno, we are in the presence of two aquifers: a river aquifer (Volturno river, the sixth largest river in Italy), and a coastal aquifer, defined as an aquifer partially or totally surrounded by the sea. This situation involves an hydraulic continuity between fresh and seawater, and - at least in the areas closest to the coast - the freshwater is supported at the base by the sea-originated saltwater penetrated inland.

Theoretically speaking, there should be a clear limit of separation (interface) between the two phases (fresh and salt water), where fresh and salt water will not mix up. Into the real world, however, such net interface does not exist; instead, there is a mixing zone where both phenomena of diffusion and dispersion are occurring (transition zone).

Salt or sea water intrusion occurs in all coastal aquifers, in which seawater flows toward inland freshwater aquifers, moving the saltwater/freshwater interface. This movement is governed by the density difference characterizing fresh and salt water. Actually, the flow moves from high-density seawater to low-density freshwater and from higher potential to lower potential (Dogan and Fares, 2008).

As it was stated several times, salinization is a quite complex process. In fact, it may occur following different dynamics. As reported by Dogan and Fares (2008), in addition to lateral intrusion from the sea, salinization processes can occur through several ways. In the literature, reference is made to "saltwater encroachment" and "sea/saltwater intrusion" - to indicate lateral and vertical movements of saltwater, respectively - and "saltwater upconing", that describes the movement of saltwater from a deeper saltwater zone upward into the freshwater zone, often favored by an extreme water pumping from the wells (Reilly and Goodman, 1987). Since those processes can act separately or concurrently, we generally tend to talk about sea/saltwater intrusion for the sake of simplicity. Finding which one of them is predominant, however, is often difficult.

Regardless of which is the preponderant dynamic, the final result is a progressive groundwater deterioration, as proved by hydro-chemical researches (Corniello *et al*., 2010). This condition is particularly delicate in Mediterranean coastal and populated areas, where excessive water demand for the population, animals, fields and factories, especially in conjunction with prolonged dry periods (Terzic *et al*., 2008), has been fostering a non-sustainable management of water resources. This causes salt stress, which is responsible for serious damages to crops and natural vegetation. Actually, salt stress inhibits plant growth by several mechanisms: it reduces the soil's water potential causing water-deficit, limits the transpiration stream (Silva *et al*., 2008) by inducing stomatal closure and causes threats to the photosynthesis machinery through an ion-excess effect (Munns *et al*., 2006; Monteverdi *et al*., 2008; Yadav *et al*., 2011). But it is well assessed that exposure to salinity can cause non-stomatal limitations too (Brugnoli and Lauteri, 1991; Monteverdi *et al*., 2008).

To address the complex issue of salinization at the mouth of the Volturno, we applied a pilot multidisciplinary study at landscape scale featuring: stable isotope analysis, leaf gas exchange measurements, geophysical investigations and NDVI calculations. This approach was chosen over others to try getting a more complete view. Actually, the different types of investigations applied complement each other, allowing us to have a more complete information set. It is inevitable, therefore, to discuss the contribution of each methodology for all the investigated aspects. Again, it should be pointed out that salt water intrusion was not addressed from mathematical/physical point of view, nor through the formulation of algorithms and models.

During our research, *Ulmus minor* Mill. was chosen as bio-indicator for salt water intrusion and secondary soil salinization for two reasons: firstly, because the genus *Ulmus* was considered moderately tolerant to salt stress according to the Water Reuse Foundation (http://salinitymanagement.org), and secondly for its presence along the SP333 roadside.

The starting point and the focus of our pilot study was the analysis of stable C and O isotopes. This dual-isotope approach has proven to be a valuable concept for ecological applications and, in particular, stable isotope technique through enriched isotope methods (Dawson *et al*., 2002) was considered the most powerful tool to investigate on plant-environment interactions and how these interactions are influenced or mediated by plants sources (water, air, nutrients). Another essential aspect is that this technique gives information on a large spatial and temporal scales range.

In this study, we focused on C and O isotopes to determine a possible seawater intrusion into fresh water, and the resulting vegetation response.

Many works based on stable isotopes analysis have been published in recent decades, confirming their high versatility and efficiency.

In particular, carbon isotope method is a good tool to investigate on plant communities physiology in coastal dunes. In fact, the ^{13}C discrimination is linked to photosynthesis via ci/ca, related to the ratio A/gs, and therefore to demand and supply of CO_2. Because of that, ΔC provides information on WUE on long term and, specifically, on the period in which the plant tissue was synthesized (Alessio *et al*., 2004, Dawson *et al*., 2002). ΔC is influenced by a series of factors, such as physiological status, genotypes, canopy structure, and environmental conditions as well.

In our study the lowest $\Delta_{dm}\,^{13}C$ and $\Delta_{ss}\,^{13}C$ values were observed in the coastal area, while higher values were observed in urban and rural area (Table 2). Regarding $\Delta_{dm}\,^{13}C$, both campaigns show the same mean values; for $\Delta_{ss}\,^{13}C$, on the other hand, there were higher mean values during October 2013, in each area. It is the first time that an isotopic study is carried out at the Volturno river's mouth and, because of that, it is very difficult to deduce certain explanations.

The behavior of the coastal area plants is mainly due to adaptation mechanisms to environmental conditions of the coastal ecosystem, like the presence of marine aerosol.

The oxygen isotope analysis allowed us to expand our knowledge on the water source used by the plant (Lauteri *et al*., 2006), since there is no isotope fractionation during water uptake by plant roots (White *et al*., 1985; Ehleringher and Dawson, 1992).

In this study, the water samples from elm xylem (Table 2), soil profiles (Table 4) and local wells (Table 3) always showed negative $\delta^{18}O$ values, indicating the continental or meteoric nature of the related aquifers.

During the second campaign, EC was measured for each well water sample (Table 3). Well #6 shows the higher EC and, at the same time, the greater abundance of the heavier oxygen isotope (Table 3). Despite these values, this well is highly exploited for corn watering. It is one of the closest to the river bed and it is not far from the elm situated at point K (Figure 5), which showed a photosynthetic activity probably reduced by non-stomatal limitations (Figure 9). In this case, a "river effect" can be hypothesized.

Focusing on the proximity of the wells (#1, #2 and #7) to the river, it is easy to note a lower concentration of the heavier oxygen isotope and divergent EC values (Figures 5, Table 3). In detail, well #1 (located by a boat hangar) showed the lowest values of EC and it is closed; well #2 (located at the city center), showed moderate EC values and it is unused too; lastly, well #7 presents a continental isotope composition but it has been recently closed for irrigation. In effect, local farmers denounced soil salinization due to a previous use of the well for watering (personal communication). This can be proved by EC values going over 1000 $\mu S\ cm^{-1}$ (Table 3).

Well #5 situation was similar to the one of well #7, with lower concentration of the heavier oxygen isotope and high EC values (Table 3). Close to well #5 there is point I, which features the most $\delta^{18}O$ - enriched values extracted from soil and the lowest resistivity minimum values determined by ERT (Table 3 and 5).

These preliminary results indicate that there are probably several factors coming into play, ie: proximity to the sea, proximity to the river and land use. We believe this last factor has a critical role, particularly in relation to the type of used water exploitation. Unfortunately, most of the farmers did not want to indicate their water management system, nor have they indicated the type of crop grown and with which rotations. Of course, those are essential information in order to get more complete investigations.

In recent years, many researches based on stable isotopes study combined with other types of analyses focused on the seawater intrusion issue were carried out.

An interesting study was carried out by Arsalan and coauthors (2012) to investigate on seawater intrusion in Bafra Plain, Turkey. In this case isopic (D, T and ^{18}O), geochemical (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Cl^{-}, SO_4^{-2}) and EC analyses were implemented too; all data were utilized to create a Piper diagram and, successively, a map to assess sea water intrusion. Comparing isotopic, physical and chemical values of well, sea and river water it was possible to define five different classes of seawater intrusion. As clearly highlighted by data, the salinization process was influenced by factors such as proximity to the river (or the sea) and wrong water management practices, as overexploitation. In this study, most of the water samples extracted from the wells were characterized by highly negative $\delta^{18}O$ values, even those considered the most saline. The salinity of those samples was found out through the association of physical-chemical parameters measurements as well. Actually, the most saline wells show higher Cl^- levels, coupled with an increase of EC and oxygen enrichment.

This element leads us to consider that, despite negative isotopic values, the salt water ingression into the aquifer cannot be excluded in our case study. Furthermore, as proposed in the next section, this aspect highlights the importance to collect sea and river water samples, in order to trace more efficiently the nature of water available to the vegetation.

A similar work based on geochemical analyses (Cl^-, SO_4^{2-}, and in particular, the ratio of $\gamma SO_4/\gamma CL$) and ^3H determination, was carried out in Laizhou Bay, Shandong Province (Zhang and Dai 2001). This kind of analysis allows determining the presence of seawater intrusion, and not only that. In effect, this methodology was also used for water dating, to distinguish between modern and quaternary water. This way, it was possible to carry out a spatial-temporal investigation on the salinization process.

Isotopic and hydro-chemical techniques gave good results in a coastal aquifer in Syria, an area characterized by over-pumping that caused an increase in salinity of shallow water. The authors (Zakhem and Hafez 2007) applied stable isotopes techniques to identify seawater intrusion. Creating the EC map, they split the area into two zones, based on the presence / absence of a mixing ratio between fresh and sea water. This EC map helps tracing the intrusion-affected zones, determining a kind of intrusion line. Moreover, the water samples were chemically and isotopically analyzed: through the comparison of deuterium, ^{18}O and Cl^-, it is possible to investigate water's nature and the presence of a mixing ratio, for each water sample.

These works show the potentiality of isotopic analysis on water sources, which allows discriminating their nature and finding out if they are subjected to saltwater intrusion phenomenon or not. In that context, our study could prove very interesting, despite being in an early stage. Following the footsteps of the mentioned studies, the isotopic analysis with physical-chemical measurements of water samples will carry an added value, enabling a quantitative and qualitative description of the ongoing phenomenon.

Focusing on gas exchange, the elm showed a significantly reduced *A* associated to both stomatal and non-stomatal limitations. Our data indicate prevailing stomatal limitations due to environment interaction in the rural area (Figure 9). Despite this, in some of the sampled points, the non-stomatal effects are particularly evident. This is the case of points K and N, where relatively low *A* values matched with high values of intercellular CO_2 concentration (C_i) (Figure 9). This occurs because non-stomatal limitations can reduce the photosynthetic capacity of the mesophyll (Brugnoli and Lauteri, 1991).

It is worth remembering that, in order to measure how efficiently plants are using water to produce biomass, we refer to WUE, estimated using two methods: through leaf gas exchange (A/gs) to evaluate intrinsic or instantaneous WUE (WUEi) or through $\Delta^{13}C$ (inversely related) to evaluate integrated WUE. In the first case, we have an information on current environmental conditions; in the second case $\Delta^{13}C$ is a well-assessed proxy of long-term WUE at plant (Farquhar *et al*., 1989; Ripullone *et al*., 2004) and ecosystem level (Scartazza *et al*., 2014). Several studies have demonstrated increases in WUE under water-stress (Ehleringher and Dowson, 1992) and stomatal limitation, via a reduction of the Ci/Ca ratio (Ripullone *et al*., 2004). While showing a similar trend (WUE and WUEi tend to increase towards the coastal zone), our results are actually characterized by very different situations from point to point, with high values in the urban part (G) and in the rural part (point J and O) as well (Figure 12).

Coupling dual-isotope approach with gas exchange measurements allows to get more information on plant physiologic status.

Values of $\delta^{18}O$ in xylem water, extracted from the same branches of the elm trees used for gas exchange measurements, were positively (although not significantly) related to $\Delta_{dm}{}^{13}C$ (Figure 11) but negatively related to the net CO_2 assimilation (Figure 10). Thus, the elm trees using relatively enriched water sources were also characterized by lower photosynthesis rates and higher $\Delta^{13}C$ values.

Vegetation status and salt-affected soils can be detected using remote sensing imagery as aerial and satellite photographs, too. Actually, remote sensing data and techniques have been progressively applied to monitor and map soil salinity since 1960s (Allbed and Kumar, 2013), assuming that a salt-affected soil shows a different surface reflectance than a salt-free soil (Shrestha and Farshad, 2012). Furthermore, unhealthy vegetation exposed to salt stress, for example, shows lower photosynthetic activity, which involves an increase of visible reflectance and a decrease of near-infrared reflectance (NIR) from the vegetation (Allbed and Kumar, 2013). Therefore, to investigate and map soil salinity, several vegetation indices (VIs) - such as Normalized Differential Vegetation Index (NDVI) and Soil Adjusted Vegetation Index (SAVI), Normalized Difference Salinity Index (NDSI) and Salinity Index (SI) - were created (Allbed and Kumar, 2013). In fact, the use of satellite imagery and the calculation of those indices are fundamental means when investigating on the vegetation physiological state, as they highlight any changes in the presence of stress factors such as salinity.

The choice of satellite imagery is often constrained by an economic factor. As in our case, we have been using medium resolution images since they are free (Landsat).

Of course, using high resolution satellite imagery could lead to more precise studies, with high predictive ability. This is the case of Allbed and coauthors (2014): using high resolution IKONOS images, they have shown that remote sensing, in combination with various statistical methods, is a powerful tool for developing soil salinity prediction models. In detail, they have been using statistical regression models based on remotely sensed indicators to predict and create a map of spatial variation in soil salinity in the Al Hassa oasis - eastern province of Saudi Arabia, which has the biggest production of date palms (*Phoenix dactylifera*) and is seriously threatened by soil salinity.

The IKONOS images include multispectral bands (blue, 0.40–0.52 μm; green, 0.52–0.60 μm; red, 0.63–0.69 μm; near-infrared (NIR), 0.76–0.90 μm) and record the reflected or emitted radiation from the Earth's surface. These bands are used to measure the Salinity Index (SI), as proposed by Tripathi *et al.*, (1997). To predict soil salinity, they compared EC with the four bands (blue, green, red and near-infrared) and SI. The correlation analysis showed a significant positive correlation ($p < 0.001$) between EC, SI and blue, green and red bands, but not with NIR band. Remotely sensed data with a significant correlation to EC were used to create a series of models. Those models providing the best fit were utilized to generate a salinity map of the study area, which shows that non-vegetated areas featured higher salinity levels. The study points out a very high prediction power (R2 = 0.65) (Allbed *et al.*, 2014).

Although, studies based on economic or free moderate resolution images (ASTER, MODIS, Landsat) still got interesting results.

In Nong Suang district in Thailand, an agricultural region characterized by high salinity distribution due to geopedological setting and salt mining activities, Shrestha and Farshad (2012) georeferenced Landsat TM data to the UTM coordinate system (zone 47) with Indian 1975 datum to investigate on salinization process. Since saline areas show higher reflectance values than non-saline areas on satellite images, they applied a rotation of red and NIR bands, in order to maximize information from soil surface. In addition, soil samples were collected for EC determination at three depths (0–30, 30–60, and 60–90 cm) and correlated with the near-surface electromagnetic (EM) induction data, a predictor for soil electrical conductivity. This method made it possible to create a map with different salinity levels, showing that nearly one-third of the area is salt affected. Moreover, this approach allows to create a salinity hazard map, especially useful to monitor areas in danger of becoming saline if no appropriate management is applied. Associating the calculation of the NDVI and NDSI indices to this approach, it is possible to conduct a detailed study about the soil salinity and the vegetation response, obtaining more complete monitoring action while keeping the costs contained.

In our study, NDVI indices - calculated using Landsat images (six data acquired by the sensors Landsat-8 ETM+ and two data by Landsat-5 TM) - give a comforting result, showing how the vegetation increased in recent years (Table 5, Figure 13). This is the result of the commendable work of citizens and the association “Le Sentinelle dei Variconi”, which takes care of the Variconi Oasis and the entire territory. But despite all, even some security constraints, there are uninterrupted acts of vandalism with frequent incidents such as arson and trees knock-down. Unfortunately, it was not possible to carry out investigations concerning the salinity status and the physiological response of the vegetation. This is partly due to our severely limited economic budget, and to the lack of collaboration by the farmers, as well. As we stand in a predominantly agricultural environment, to make a detailed and reliable analysis, it would be desirable to know the land use and cultivation target for every single field, in order to know the NDVI time series trend for a comparable reference.

Hopefully, in the upcoming future, we will close new agreements with local governments, which will allow us to access more information on both the current situation and historical data. Our priority will be focusing on remote sensing techniques, also taking inspiration from the studies mentioned above.

The last part of our multidisciplinary pilot study focused on geophysical investigations, through the production of ERT sections that provided information on the presence of salt or brackish water into the aquifer.

The low-resolution ERT profiles in the three monitored areas, gathered using electrodes spaced by 4, 5 or 10 m, give us preliminary information on possible sea water intrusion. The results indicate the presence of salt and brackish water at about 2 m from ground level in the coastal area: this is proved by the presence of *Salicornia* and *Juncus genera*, which are typical vegetation of saline soils. Instead, in urban and rural areas, salt and brackish water was found at a depth of about 13-15 m (Figure 14). These depths do not rouse troubles to vegetation and crops. Although there were no alarming signs of water salinization, the testimonies of the farmers led us to intensify the investigations within the agricultural area. The high-resolution surface ERT (Figure 15) acquired on July 2014 in rural area, actually, revealed low resistivity values at depths of 0.5 and 1 m (3-15 Ωm), revealing the presence of a shallow groundwater system featuring a moderate concentration of salts.

Thus, while the large-scale ERT analysis indicates a reduced risk of secondary salinization for the soil water (because of the fresh / salt water interface at relatively high depths), the high resolution ERT sections show the presence of a surface salinity due to small-sized clayey layers, which were not highlighted in the profiles with the electrodes spaced by 4 m and more.

In the same study area, Carrara *et al*., (2005) showed a significant lateral variation of resistivity within the aquifer using electro-seismic models. Authors found lens with smaller resistivity in the range between 0.8 and 1.0 Ωm, addressing these values to the presence of clay lens impregnated with salt water.

Recently, Corniello *et al*. (2010) carried out a study to draw up a hydrogeological map of the neighbour area of the Volturno mouth. In this area, the complex lithology resulted characterized by pyroclastic/alluvial/marine deposits, with a variable permeability ranging from low to medium-high. The authors came to the conclusion that saltwater intrusion is generally limited along the coast, but it becomes more relevant along the last part of the river, probably due to a) intrusion of denser seawater into the riverbed and b) links between riverine and groundwater aquifers. In fact, the upstream rise of saline water along the river may occur because the Volturno bed lies about 3.5 -3.7 m below the sea level. This is true up to the locality of Cancello Arnone, approximately 10 Km inner from the mouth.

These two researches (Carrara *et al*., (2005) and Corniello *et al*. (2010)) carried out in our study area in a fairly recent period are very important, as they are partly a confirmation of our preliminary results and partly an encouragement to move forward with our analysis.

As it was pointed out several times in the previous chapters, this research is a pilot study, meaning it is designed to set up a multidisciplinary methodology, considered the only viable alternative to deal with a complex issue such as is the salinization of water resources. That is because, most of the times, several factors are simultaneously contributing to the process. Another point that is worth mentioning is that often registered signals may be due to different causes. Especially in this case, a 360°analysis is particularly appropriate.

In recent decades the increase of population density - especially in coastal areas - and the consequent increasing water demand for domestic, agricultural and industrial purposes, led to a gradual increase of salinization risk, and this risk will keep growing.

Once perfected, this methodology can be applied to other case studies showing similar problems. That is the importance of an experimental study: setting up a method to deal with the studied issues (also at different spatial and temporal scales), using all necessary modifications and adjustments based on the detected environmental characteristics.

In particular, our attention is headed towards the Mediterranean basin, in which a considerable amount of critical situations is occurring. Several studies about the seawater intrusion phenomenon were carried out along the whole Italian peninsula, especially in coastal areas of Veneto, Tuscany, Sardinia, Campania and Puglia, where tourism activities (along with the presence of rural areas and, often, industrial complexes and a fairly high population rate), leads to increasing water demand which often causes unsustainable management of water resources.

In detail, at Versiliese-Pisan coastal aquifer (Tuscany), Doveri *et al*. (2010) carried out a study based on water level measurements and physical (electric conductivity, temperature, pH), chemical (Cl^-, SO_4^{2-}, Br^-, NO_3^-, Na^+, K^+, Ca^{2+}, Mg^{2+}) and isotopic analysis ($\delta^{18}O$‰ and $\delta^{2}H$‰) of the water sampled in wells/piezometers and in the main streams. Actually, they made hydrostratigraphic correlations, showing that the higher EC values (up to 17,000 μS/cm) were found in the low water level zones and close to the coastline, and they were characterized by Cl-Na composition. Furthermore, some groundwater and surface water samples were characterized by higher values of both EC and $\delta^{18}O$‰. Being this study conducted within the Migliarino - S.Rossore - Massaciuccoli Regional Park, we believe it would be very interesting to expand it on the ecophysiological part as well, in order to analyze the vegetation responses in relation to the used water's quality. Finally, a remote-sensing investigation could give an overview of the physiological status and geophysical methods may help in the investigation of the aquifer's quality.

Another interesting application of geophysical methods joint with physical and hydrochemical analysis was conducted in Sardinia, at alluvial plain of Quirra and Flumini Pisale rivers (Sodde and Barrocu, 2006), where the determination of the seawater intrusion phenomenon is combined with the variation of heavy metal concentrations. The results indicate that metals react to one or more retoxification factors, all depending on aquifer salinization dynamics, proving as this type of applications can also be useful to monitor the water deterioration not only due to the salt concentrations, but also to heavy metals. Again, we think that expanding the study with an ecophysiological analysis could help to have a full picture of the situation.

In this regard, we believe that, once improved, our methodology could be applied to the investigation of salt water intrusion, in the most sensitive ecosystems around the world showing the combination of several critical factors. Of course, the approach would be adjusted and customized depending on the ecosystem and the study area's features. Therefore, it would support hydrological, geological or physiological investigations, according to the situation in place.

Today, approximately 3 billion people, corresponding to slightly less of half the world's population, live within 200 kilometers of a coastline. By 2025, that figure is likely to double. The problem is particularly acute in developing countries (Creel, 2003).

Statistics (http://ec.europa.eu/eurostat/statistics-explained/index.php/Coastal_regions_-_population_statistics) show some disparities in population distribution patterns along the European coastline. Actually, the 439 coastal regions of the European Union (EU), which cover only the 40.0 % of EU-27 territory, are populated by 40.8% of the total inhabitants. In the Mediterranean countries, the population grew from 276 million in 1970 to 466 million in 2010, and population in the coastal regions grew from 95 million in 1979 to 143 million in 2000. In fact, more than a third of inhabitants live in coastal areas, even though they represent less than 12 % of the Mediterranean territory (http://www.grida.no/graphicslib/detail/population-density-and-urban-centres-in-the-mediterranean-basin_1a99).

In the rest of the world, especially in Asia, the percentages are getting even more extreme. China is the most populated nation in the world. The population is condensed in the eastern part of the country, especially in the coastal zones. Actually, nearly 115 million people (or 11% of the population) live in an area covering 0.5% of China's total land area, and 1 billion Chinese (or more than 90% of the population) live in only a little more than 30% of China's territory. The population density of this area is 354 people per square kilometer (http://cgge.aag.org/PopulationandNaturalResources1e/CS_China_July09/CS_China_July094.html).

The main cause of the groundwater salinization phenomenon lies in these numbers: they show the importance of starting monitoring actions to address the depletion of water quality through sustainable use of water resources and implementing ecological water management practices.

Of course, this kind of study has also an economic impact. Being coastal areas highly populated, they are also characterized by the presence of strong economic activity in both agriculture and industry. The salinization problem would damage the whole area, affecting the productivity of the fields and, consequently, of the animals. The decline in agricultural productivity would have a domino effect on food industries that deal with the transformation of the products. The same applies to the productivity of livestock. Being in a territory where the agricultural and industrial sectors are fundamental for the city’s economy, it is easy to understand how necessary it is to intervene as soon as possible in order to protect the economy itself, maybe drawing attention of foreign stakeholders to invest in typical and quality products.

6 Conclusions

This pilot work tries to bridge the gap between research and territorial policies and comes in response to a request of the farmers, who have noticed an increasing salinization of the soils and a consequent loss of productivity in recent years.

In primis, it is important to remember that the study area is damaged by micro and macro criminality, abandoned by the political authorities, characterized by a complex social fabric and located near one of the most polluted sites of Italy, “Terra dei Fuochi". These aspects have been a strong limit to the research activities, which were forced to face bureaucratic and budget-related issues, and also with the locals’ lack of collaboration and opposition.

The study is based on a multidisciplinary approach that allowed to highlight a complex situation, still not entirely clear. Referring to this approach, none of the techniques *per se* is able to provide a clear picture of the salinization risk at the landscape scale. But as a whole, each investigation technology revealed some aspects related to salt presence in some of the ecosystem compartments.

Starting from the territorial structure, from air photos, it is clear that there has been a substantial loss of soil along the coastline during the years. Nevertheless, this phenomenon seems to have slowed down in recent years, thanks to the implementation of an anti-erosion barrier, which, however, seems not to be fully working, because of some installation problems (information obtained through interviews to the locals). In recent years, there has been an increase in vegetation, as well.

The data gathered joining the geophysical and ecophysiological approaches suggest there is not a relevant salinization of the water aquifers in the area at the moment, at least related to a massive intrusion of marine water. However, we observed a series of warning signals by analysing high resolution ERTs, plant physiological status and stable isotope hydrology.

The three negative, significant and linear relationships between $\delta^{18}O$ and *A*, *gs*, and ΦPSII are particularly important, as they indicate that enriched water affects photosynthesis, stomatal aperture and electron transport efficiency. The decrease of *A* in the rural area suggests that the different land-uses play an important role. Actually, photosynthetic activity seems limited by stomatal and non-stomatal limitations. In the latter case, either drought and salinity could be responsible.

Through the high-resolution ERT, the presence of saline – salty/brackish – brackish water has been proven. Referring to the first 3m of soil depth, the data is quite worrying for the vegetation *status*.

To irrigate the fields, the farmers are pumping water from wells, often at excessive rates, also because of drought periods in the summertime. In the long run, that may cause a rise of saltwater which, although not fully reported by our data yet, has already been denounced by the farmers themselves, who proceeded to close and abandon some wells.

Furthermore, some data and previous studies suggest that the saltwater risk seems linked to the lateral intrusion in the river's meanders.

Further studies should focus on the hydrological dynamics at the interface between the river water and the underground water as well as on the effect of irrigation water on crops physiological performances. Also, a map of the salinization risk should be traced.

Although this is a pilot study, it is quite important for setting up a methodology. This is only the first step, however. We would like to continue developing and extending it according to the following points:

- Extending isotopic analysis to deuterium as well;
- Intensification of sampling points along the transect;
- Carrying out isotopic analysis of plant and soil samples taken in every single point of the transect, and measuring the photosynthetic activity of each helm;
- Isotopic analysis of river water at different depths and along the transect, from the mouth to the inner part;
- Isotopic analysis of sea water;
- Chemical analysis of water extracted from soil and twigs;
- Areas characterization according to the different land-uses;
- Intensification of remote sensing investigation.

Concurrently, an active exchange of information between researches and territorial actors is urgently needed, to establish a direct dialogue to mitigate any risky land use and to operate the proper planning and management actions. Indeed, simple and cost-effective applications of hydraulic engineering could largely solve the problem on a large territorial scale. A proper maintenance of the riverbed and the setup of infrastructures, like small submersed dams or an expansion of the hydraulic section, could limit the saline counter flow entering the riverbed.

We hope that this study will go on, keeping the use of a multidisciplinary approach for wide-ranging investigation on a complex topic such as the saltwater intrusion and the resulting responses of vegetation. For those reasons, we hope for more scientific studies and monitoring activities, for the sake of this territory and its people. Such simple applications would guarantee the viability of the coastal ecosystems and the land use sustainability, with important socio-economic implications.

Finally, as already stated, this study and its methodology, once consolidated, can be applied to other case studies showing similar problems. In particular, our attention goes to the Mediterranean basin, where the high population rate - associated with higher incidence of environmental forcing - compromises the water resources.

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8 List of figures and sources

Figure 8: Relationships between Δ_{dm} and Δ_{ss} during October 2013 and July 2014. Each point represents a different site located along the study transect from the mouth of the Volturno river to the inner agricultural area. Each value represents the average of three replicates. The values of the correlation coefficient (r) and the significance level (P) of the linear regressions are shown.

Figure 9: Net CO_2 assimilation rate (A) and stomatal conductance (g_s) (upper panel), internal CO_2 concentration (C_i) and transpiration rate (E) (middle panel) and actual photochemical efficiency of photosystem II (Φ_{PSII}) (lower panel) determined during July 2014 in different point of the study transect (see Figure 3). Values represent the average of three replicates ± standard error (SE). Data were analyzed independently by one-way ANOVA. For each parameter, data followed by different letters are significantly different ($P \leq 0.05$, Student–Newman–Keuls).

Figure 10: Relationships of oxygen isotope composition ($\delta^{18}O$) of xylem water with CO_2 assimilation rate (A, upper panel), stomatal conductance (g_s, middle panel) and actual photochemical efficiency of photosystem II (Φ_{PSII}, lower panel). Each value represents the average of three replicates ± standard error (SE). The letters indicate different points along the study transect (see Figure 3). The values of the correlation coefficient (r) and the significance level (P) of the linear regressions are shown.

Figure 11: Relationships between carbon isotope discrimination of leaf dry matter (Δ_{dm}), actual photochemical efficiency of photosystem II (Φ_{PSII}; upper panel) and oxygen isotope composition ($\delta^{18}O$) in xylem water (lower panel). Each value represents the average of three replicates ± standard error (SE). The letters indicate different points along the study transect (see Figure 3). The values of the correlation coefficient (r) and the significance level (P) of the linear regressions are shown for both for all data and only for the rural area.

Figure 12: Variation of intrinsic WUEi (A/gs - continuous black line) and Δ_{DM} ^{13}C (inversely related to long term WUE - dashed grey line) along the transect.

Figure 13: Panel a) shows a NDVI picture, taken in 2013. The values are referred to: a dense vegetation canopy (from 0,3 to 0,8), soil (from 0,1 to 0,2) and clear water (barely positive or even slightly negative values). The panels b) and c) show a comparison of vegetation cover between 2013 and 1984 - 2003, respectively. Created by Maurzio Sarti.

Figure 14: Low resolution Electrical Resistivity Tomography (ERT) profiles carried out during 2013 at coastal (Profile1), urban (Profile 2) and agricultural areas (Profile3). Created by Daniela Tarallo.

Figure 15: High resolution Electrical Resistivity Tomography (ERT) profiles carried out on July 2014 in different points of the rural area along the study transect (see Figure 5). Created by Daniela Tarallo.

9 List of tables and sources

Table 1: Resistivity of water and sediments, proposed by Zohdy *et al*., 1993.

Table 2. Carbon isotope discrimination of both leaf dry matter (Δ_{dm}) and leaf soluble sugars (Δ_{ss}) and oxygen isotope composition ($\delta^{18}O$) in xylem water determined along sixteen points (from A to O) along the study transect during October 2013 and July 2014 sampling campaigns. The first part of the transect (points A-B-C-D) is within the coastal area (Cs), the second part (points E, F, G) is within the urban area (Ru) while the third part (points H, I, J, K, L, M, N, O) falls in the rural area (Ru). The average values ± standard error (SE) of the whole transect and of the three different areas for 2013 and 2014 sampling campaigns are shown (n.d., not determined). ** or * on the average values indicate, respectively, highly significant or significant difference of the specific mean value, in respect to the other means in column.

Table 3. Oxygen isotope composition ($\delta^{18}O$) of water from wells, sampled in October 2013 and July 2014. Each value represents the average of three replicates ± SE (n.d., not determined). The values of pH and electrical conductivity (EC) were measured only on pooled water samples collected on July 2014, at the standard condition of 25°C. For the July sampling data is reported also the $\delta^{18}O$, pH and EC values of the tap water.

Table 4. Oxygen isotope composition ($\delta^{18}O$) of water extracted from soil samples taken at three depths (40, 60 and 80 cm) in different sites along the study transect, during the July sampling campaign. Each value represents the average of three soil samples for each depth. The average values ± standard error (SE) for different depth are shown (n.d., not determined).

Table 5. NDVI estimation of gain and loss in vegetation for year 2013 compared to years 1984 and 2003.

Table 6. Minimum resistivity values determined by the Electrical Resistivity Tomography (ERT) sections carried out on July 2014 in different sites of the rural area along the study transect. (see Figure 1 and supplementary material).

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