UNIVERSITÀ degli STUDI di CATANIA | Dipartimento di Agricoltura, Alimentazione e Ambiente
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UNIVERSITÀ DEGLI STUDI DI CATANIA

AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCE
XXXIII CICLO

Biomass Crops in Marginal Lands

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Ph. D. attended during 2017/2020

Summary

Abstract

In recent years, a debate has emerged regarding food security and land use for bioenergy/industrial non-food crops. Cultivating biomass crops on marginal land unsuitable for food production is consistently proposed as an alternative to minimize iLUC effects about land-use competition for food production, and its adverse effects (direct or indirect) on food security, land based GHG emissions and biodiversity loss.

Several studies agree on the existence of a considerable extension of land in Europe less suitable for conventional agriculture. This land has been either abandoned for its low productivity, or it is used as grassland. No-food biomass crops can provide abundant renewable feedstocks for the production of high added-value bio-based commodities and bioenergy, thus feeding the bio-based economy.

Nowadays, the cultivation of biomass crops on marginal land to avoid land-use competition with food production is a central debate; therefore, this study aims to promoting the sustainable development of resource-efficient and economically profitable lignocellulosic biomass crops grown on soil affected by salinity, which is considered a biophysical constraint leading to cultivation of bioenergy crops on marginal lands.

The present experiment focused on lignocellulosic crops that grow naturally in the Mediterranean environment (*Arundo donax*, and *Saccharum spontaneum*), and the leading perennial bioenergy grass (Miscanthus *x giganteus*) in order to provide information on their tolerance to salinity stress. Furthermore, the use of fertilization as a further experimental factor was employed, with the aim to ascertain a possible

sensitivity mitigation to salinity stress. Two independent experiments were carried out, named Experiment 1 (throughout 2019 growing season) and Experiment 2 (throughout 2020 growing season), both involving perennial bioenergy grasses under soil salinity and fertilization levels in pots amounting to 10 kg of soil each. In Experiment 1, rhizomes were used to propagate perennial grasses, which were grown in a clay soil under open air. In Experiment 2, stem node cuttings were used to propagate perennial grasses, the soil was of volcanic origin and crops were grown under semi-open air in a glasshouse which was open in the four sides. Both substrates were differentiated since representatives of two different types of marginal soil in south-eastern Sicily (Italy). In both Experiment 1 and Experiment 2 the three different species, three levels of NaCl (S_0, S_1 and S_2, respectively 0, 9 and 18 dS m^{-1}) and three levels of NPK fertilizer (F_0, F_1 and F_2, respectively 0, 60 and 120 kg NPK ha^{-1}), were replicated three times in a completely randomized experimental design.

During the crop cycle and at harvest, the soil electrical conductivity (EC), physiological (net photosynthesis, stomatal conductance, transpiration rate and instantaneous water use efficiency, chlorophyll fluorescence), morphological (height of main stem, leaf area index, number of green and dry leaves), productive (above and belowground dry biomass) and quality traits (protein, hemicellulose, cellulose, ADL and ash) were measured.

The soil salinity concentration, on average for the three species per single treatment, increased during the growth cycle. In all treatments the initial EC increased approximately to 5 dS m^{-1} in S_0, 20 dS m^{-1} in S_1 and 25 dS m^{-1} in S_2 after 1 month of treatment with NaCl. Soil EC also increased in the untreated treatment due to the effect of the salt concentration in the irrigation, medium brackish, water.

In Experiment 1, main findings highlighted the high tolerance to salinity of the investigated perennial grasses. While morphological and physiological traits throughout the experimental period were someway affected, there was a not clear trend with the treatments imposed to the crops, although main effects were statistically significant. On the other hand, biomass productivity at harvest showed a higher biomass production for *Arundo* and *Saccharum* than for *Miscanthus*. Salinity followed a gradient, decreasing as the salinity level increased: the biomass production between the salinity control (S_0) and the medium salinity level (S_1), across fertilizations, reduced by 21% in *Arundo*, 59% in *Saccharum* and 63% in *Miscanthus*. The reduction between S_0 and the highest salinity level (S_2) was 38% in *Arundo*, 60% in *Saccharum* and 70% in *Miscanthus*.

Fertilization had a beneficial effect in the different salinity levels and crops. As expected, the highest fertilization clearly improved the biomass production in all crops under not saline environments. The biomass quality in Experiment 1 was carried out for hemicellulose and cellulose content on stems and leaves. The soil salinity increase lead to a linear decrease of hemicellulose content both on leaves and stems, while increasing the fertilization increased hemicellulose on leaves but not in stems. Among species, *Miscanthus* and *Saccharum* had a significantly higher hemicellulose content than *Arundo* in leaves, while *Saccharum* and *Arundo* on stems. *Arundo* showed also the significantly highest cellulose content on stems, followed by *Miscanthus* and by *Saccharum*. Also, in this case, increasing the salinity level decreased significantly the cellulose content on stems. The fertilization decreased the cellulose content on stems but was ineffective on leaves.

In Experiment 2 an inverse response of morphological (stem height and leaf area index) and productive traits (stems, leaves and root dry weight) to increasing salinity levels was also observed. Increasing fertilization levels with NPK increased the main morphological and productive traits mitigating, in part, the salinity stress. Among species, *Saccharum* was the tallest, *Arundo* had the highest LAI, *Miscanthus* the highest leaf and root dry weight, while *Arundo* the highest stem dry weight.

As expected, the C3 *Arundo* showed the significantly highest stomatal conductance, which lead to a higher transpiration rate among species. The two C4, *Saccharum* and *Miscanthus,* although did not differ their stomatal conductance showed a significantly different instantaneous water use efficiency, with *Saccharum* having the highest overall, however, both were more efficient than *Arundo*. The salinity stress reduced both stomatal conductance and the instantaneous water use efficiency, while the fertilization increased both parameters, but the significant effect was observed only for the stomatal conductance. On the other hand, the maximum efficiency of photosystem II was quite similar among the C3 and the C4 crops. The fertilization increased slightly this trait, while the salinity stress had a strong, depressing effect on the maximum efficiency of photosystem II. It is worth to mention that *Arundo* seems to be less affected to harsher conditions, reducing this trait to a lesser extent as compared to the other species.

The biomass quality was affected to a different extent in relation to the treatments, species and plant part. Generally, leaves had a higher amount of protein and ash than stems,

while stems had a higher amount of cellulose and ADL. The fertilization increased both protein and ash in leaves and stems, but the structural compounds (i.e., hemicellulose, cellulose and ADL) were less affected. The increase in salinity decreased the protein and increased the hemicellulose on leaves, while increased the ash on stems. The other biomass compounds were not significantly modified by salinity. Among species, *Arundo* had the highest protein and ash, both on stems and leaves, the hemicellulose was the highest in *Saccharum* leaves and in *Miscanthus* stems, the cellulose was the highest in *Saccharum* and *Miscanthus* leaves, while the three species had a similar cellulose amount on stems. The ADL was the highest in *Miscanthus* and *Arundo* and the lowest in *Saccharum*, both stems and leaves.

Sommario

Negli ultimi anni è emerso un dibattito sulla sicurezza alimentare e sull'uso del suolo per tutte quelle colture definite bioenergetiche/industriali non alimentari. La coltivazione di colture da biomassa su terreni marginali e quindi inadatti alla produzione alimentare viene proposta come una valida alternativa per ridurre al minimo gli effetti iLUC relativi al cambiamento indiretto della destinazione d'uso del suolo per la produzione alimentare e i suoi effetti negativi (diretti o indiretti) sulla sicurezza alimentare, sulle emissioni di GHG (gas ad effetto serra) nell'atmosfera e sulla perdita di biodiversità.

Diversi studi hanno dimostrato la presenza di una notevole estensione territoriale in Europa di suoli meno adatti all'agricoltura convenzionale. Questi terreni sono stati

abbandonati per la loro bassa produttività e vengono utilizzati prevalentemente per il pascolo. Le colture da biomassa non alimentari possono fornire abbondanti produzioni di materie prime definite bioenergetiche rinnovabili ad alto valore aggiunto, incrementando così lo sviluppo di una nuova economia definita "bio-based" che si basa sull'uso di materiali o prodotti che siano interamente o parzialmente derivati da biomassa quali le piante e i vegetali in genere.

Al giorno d'oggi, la coltivazione di colture da biomassa su terreni marginali, per evitare la competizione per l'uso del suolo con le produzioni alimentari, rappresenta un dibattito di notevole attualità. Pertanto, questo studio mira a promuovere lo sviluppo sostenibile delle colture da biomassa di origine lignocellulosica, efficienti per l'utilizzo delle risorse ed economicamente redditizie, coltivate su terreni colpiti da salinità, che è considerato un vincolo biofisico per la coltivazione di colture bioenergetiche su terreni marginali.

Il presente lavoro di ricerca si è concentrato sulle colture lignocellulosiche che crescono spontaneamente nell'ambiente mediterraneo (*Arundo donax* e *Saccharum spontaneum*) e su una coltura erbacea perenne diventata leader tra le colture bioenergetiche (*Miscanthus* x *giganteus*) con lo scopo di fornire informazioni sulla loro tolleranza allo stress da salinità. Inoltre, è stato impiegata la tecnica della fertilizzazione come ulteriore fattore sperimentale, con l'obiettivo di accertare una possibile mitigazione della sensibilità allo stress da salinità. Sono stati condotti due esperimenti indipendenti denominati Esperimento 1 (durante la stagione di crescita del 2019) ed Esperimento 2 (durante la stagione di crescita del 2020). In entrambi i casi sono state utilizzate colture erbacee bioenergetiche perenni allevate in vasi con circa 10 kg di terreno ciascuno e sottoposte a diversi livelli di salinità del suolo e diversi livelli di fertilizzazione. L'esperimento 1 è stato condotto ponendo i vasi, ripieni di terreno argilloso, in pieno campo. Come materiale di propagazione per le colture erbacee perenni sono stati scelti i rizomi. L'esperimento 2 è stato condotto ponendo i vasi, ripieni di terreno di origine vulcanica, in pieno campo all'interno di una serra aperta sui quattro lati. Come materiale di propagazione per le colture erbacee perenni sono state scelte le talee nodali prelevate dai culmi. Sia nel primo che nel secondo esperimento i substrati (argilloso–vulcanico) sono stati differenziati perché sono rappresentativi di due diverse tipologie di suolo marginale presenti nella Sicilia sud-orientale (Italia). Nell'Esperimento 1 e nell'Esperimento 2, delle tre diverse specie scelte, i tre livelli di NaCl (S_0, S_1 e S_2, rispettivamente 0.9 e 18 dS^{m-1}) ed i tre livelli di fertilizzante NPK (F_0, F_1 e F_2, rispettivamente 0.60 e 120 kg di NPK ha^{-1}), sono stati replicati tre volte seguendo un piano sperimentale completamente randomizzato.

Durante il ciclo colturale e alla raccolta, sono stati elaborati i dati relativi alla conducibilità elettrica del suolo (EC), i dati fisiologici (fotosintesi netta, conduttanza stomatica, tasso di traspirazione ed efficienza dell'uso istantaneo dell'acqua, fluorescenza della clorofilla), i dati morfologici (altezza del fusto principale, indice dell'area fogliare, numero di foglie verdi e secche), i dati produttivi (biomassa secca sia della parte epigea che della porzione ipogea delle singole piante) ed i dati relativi alla qualità della biomassa (proteine, emicellulosa, cellulosa, ADL e ceneri).

La concentrazione della salinità del suolo, mediamente, per le tre specie in ogni singolo trattamento, è aumentata durante il ciclo di crescita. In tutti i trattamenti l'EC iniziale è aumentata dopo 1 mese di trattamento con NaCl approssimativamente a 5 dS m^{-1} in S_0, 20 dS m^{-1} in S_1 e 25 dS m^{-1} in S_2. La EC del suolo è aumentata anche nelle tesi di controllo (S_0) per effetto della concentrazione del sale presente nell'acqua usata per l'irrigazione, che dalle analisi effettuate è risultata mediamente salmastra.

Nell'esperimento 1, i risultati principali hanno evidenziato l'elevata tolleranza alla salinità delle colture erbacee perenni studiate. Mentre i tratti morfologici e fisiologici durante tutto il periodo sperimentale sono stati in qualche modo influenzati dai trattamenti imposti alle colture, gli effetti principali sono risultati statisticamente significativi. D'altra parte, la produttività della biomassa, alla raccolta, ha mostrato una produzione di biomassa maggiore per *Arundo* e *Saccharum* rispetto a *Miscanthus*. La salinità ha seguito un gradiente, infatti con l'aumentare del livello di salinità diminuivano i seguenti parametri: la produzione di biomassa, tra il livello di controllo della salinità (S_0) e il livello di salinità medio (S_1), durante le concimazioni, è diminuita del 21% in *Arundo*, del 59% in *Saccharum* e del 63% in *Miscanthus*. La riduzione in termini di biomassa tra il livello di controllo (S_0) e il livello di salinità più alto (S_2) è stata del 38% in *Arundo*, del 60% in *Saccharum* e del 70% in *Miscanthus*.

La fertilizzazione ha avuto un effetto benefico sia nei diversi livelli di salinità sia nelle colture. Come previsto, la fertilizzazione più elevata ha chiaramente migliorato la produzione di biomassa in tutte le colture in terreni non salini. La qualità della biomassa nell'esperimento 1 è stata rilevata, in termini di contenuto di emicellulosa e di cellulosa, sui culmi e sulle foglie. L'aumento della salinità del suolo ha determinato una diminuzione lineare del contenuto di emicellulosa sia sulle foglie che sui culmi, mentre aumentando la fertilizzazione va ad aumentare l'emicellulosa sulle foglie ma non sui culmi. Le specie, *Miscanthus* e *Saccharum*, hanno mostrato un contenuto di emicellulosa significativamente più alto nelle foglie, mentre il *Saccharum* e l'*Arundo* sui culmi. L'*Arundo* ha mostrato anche un contenuto di cellulosa significativamente più alto sui culmi, seguito da *Miscanthus* e *Saccharum*. Anche in questo caso, aumentando il livello di salinità è diminuito sensibilmente il contenuto di cellulosa sui culmi. La fertilizzazione ha diminuito il contenuto di cellulosa sui culmi, mentre non ha influito sui parametri qualitativi della biomassa fogliare.

Nell'esperimento 2 è stata osservata anche una risposta inversa, dei dati morfologici (altezza del culmo e indice dell'area fogliare) e di quelli produttivi (culmi, foglie e peso secco dell'apparato radicale), all'aumentare dei livelli di salinità. L'aumento dei livelli di fertilizzazione con NPK ha aumentato i principali parametri morfologici e produttivi mitigando, in parte, lo stress da salinità. Tra le specie, il *Saccharum* era quello con i culmi più alti, l'*Arundo* aveva il LAI più elevato, il *Miscanthus* mostrava il più alto peso secco sia delle foglie sia dell'apparato radicale, mentre l'*Arundo* mostrava il più alto peso secco dei culmi.

Come previsto, la specie C3 l'*Arundo* ha mostrato il dato della conduttanza stomatica significativamente più elevato, questo ha determinato anche un tasso di traspirazione più elevato tra le varie specie prese in esame. Le due specie C4, il *Saccharum* e il *Miscanthus*, sebbene non differissero per la loro conduttanza stomatica, hanno mostrato un'efficienza di utilizzo istantaneo dell'acqua (iWUE) significativamente diversa, infatti il *Saccharum* ha mostrato il più alto tasso iWUE. Tuttavia, entrambe le specie C4 si sono dimostrate più efficienti rispetto all'*Arundo*. Lo stress da salinità ha ridotto sia la conduttanza stomatica che l'efficienza dell'uso istantaneo dell’acqua (iWUE), mentre la fertilizzazione ha determinato un aumento di entrambi i parametri, ma l’effetto più significativo è stato osservato solo per il parametro della conduttanza stomatica. D’altra parte, la massima efficienza del fotosistema II era abbastanza simile tra le colture C3 e C4. La fertilizzazione ha leggermente aumentato questo parametro, mentre lo stress da salinità ha avuto un effetto negativo, riducendo drasticamente l’efficienza del fotosistema II. È importante ricordare che l’*Arundo* ha mostrato una maggiore resistenza e rusticità in condizioni di stress estremo, riducendo questo parametro in misura minore rispetto alle altre specie.

La qualità della biomassa è stata influenzata in misura diversa in relazione ai trattamenti, alle specie e agli organi delle piante. In generale, l’apparato fogliare ha mostrato una quantità maggiore in termini di proteine e di ceneri rispetto ai culmi, mentre i culmi hanno mostrato un contenuto maggiore di cellulosa e ADL. La fertilizzazione ha aumentato sia le proteine che le ceneri nelle foglie e nei culmi, invece i composti strutturali (emicellulosa, cellulosa e ADL) sono stati meno influenzati dalla fertilizzazione. L’aumento della salinità ha determinato una diminuzione del tasso di proteine ed un aumento del tasso di emicellulosa nell’apparato fogliare, al contrario ha determinato un aumento del contenuto in ceneri nei culmi. Le altre componenti della biomassa non sono state modificate in modo significativo da un aumento del tasso di salinità nel terreno. Tra le varie specie prese in esame, l’*Arundo* ha mostrato il più alto tasso di proteine e di ceneri, sia sui culmi che sulle foglie, nelle foglie di *Saccharum* e nei culmi di *Miscanthus* sono stati rilevati alti tassi di emicellulosa, al contrario è stato rilevato un tasso molto elevato di cellulosa nelle foglie di *Saccharum* e di

Miscanthus; comunque, le tre specie hanno mostrato un tasso di cellulosa abbastanza simile sui culmi. L'ADL è stato il parametro con il tasso più elevato nel *Miscanthus* e nell'*Arundo* e il più basso nel *Saccharum*, sia nei culmi che nelle foglie.

Introduction

During the first industrial revolution that began in England between 1760 and 1780, according to the English historian Thomas Southcliffe Ashton, fossil fuels such as coal were used for the first time and it was widely believed that they were an inexhaustible source of energy.

However, in 1970, the global energy and financial crisis prompted governments to seek alternative energy sources called "renewable" compared to fossil fuels defined "non-renewable" and promote their use.

In 1979 took place the first World Climate Conference and the World Climate Program was established, global warming caused by increased greenhouse gas emissions into the atmosphere was discussed (CO_2) and the thinning of the ozone layer (O_3) which performs the essential function of protecting the earth's surface from overheating.

Subsequently, the guiding objective of the various United Nations Conferences that took place from the 70s to today was the need to find alternative or "renewable" energy sources that could replace fossil fuels such as coal, the source of non-renewable energy.

For these reasons, an intergovernmental group on climate change and the United Nations Environment Program were created in 1988.

The United Nations Conference on Climate also called the "Earth Summit" was held in Rio de Janeiro in 1992, which led to the drafting of a document known as Agenda 21.

Obviously, it was a non-binding action plan, which could be voluntarily implemented by the states that had participated in the United Nations Conference on Climate to encourage sustainable development.

The United Nations Framework Convention on Climate Change (UNFCCC) entered into force on March 21, 1994.

However, only in 1997 did the UNFCCC become operational with an international agreement, the so-called "Kyoto Protocol" which set common goals among participating States to reduce global greenhouse gas emissions.

The common rules to be adopted by each state were established during the Conference of the Parties (COP7) in Marrakech in 2001 and were called the "Marrakech Agreements".

The aim was to reduce greenhouse gas emissions by 5% from 1990 levels over a five-year period from 2008 to 2012, for the 37 participating industrialized countries including the European Union (EU-15).

The Kyoto Protocol entered into force during the first meeting of the CMP1 and COP11 parties which took place in Montreal on 16 February 2005.

In 2015, COP21 and CMP11 were held in Paris and the urgency of implementing the Kyoto Protocol convention to limit the increase in the earth's temperature and promote the use of sustainable and renewable energy was also underlined of development.

The Paris Agreement entered into force on 5 October 2016 and the formal agreement was reached on 4 November 2016. It is important to note that not all Member States that participated in the Paris Agreement signed it[1].

European Community policy strongly supports the Kyoto Protocol and to reduce its dependence on imported oil and petroleum products, thus improving the security of energy supplies in the medium and long term[2] and proposes an increasing use of biomass crops for energy as a key tool for reducing greenhouse gas emissions.

The use of biomass crops for energy on a global scale could help improve the environment because the biomass sources are also defined as "carbon neutral" in fact, the carbon they emit into the atmosphere is compensated by the carbon that plants absorb from the atmosphere[3].

Various biomass raw materials for energy have been produced in the European Union [4], such as those from arable crops and also from dedicated crops the so-called “energy crops”, from forestry, from household waste, etc.

The most promising sources of biomass for energy are dedicated lignocellulosic crops which are used for the production of heat and electricity through direct combustion or for the production of biofuels and biogas (Yang and Wyman, 2008).

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[1] http://unfccc.int/paris_agreement/items/9485.php

[2] European Biofuels Technology Platform, 2008.

[3] Royal Society, 2008.

[4] From EU Directive 2001/77 and Legislative Decree 387/2003, modified by EU Directive 2009/28 and Legislative Decree 28/2011, the term biomass must be understood as “the biodegradable fraction of products, waste and residues of biological origin from agriculture (including plant and animal substances), forestry and related industries, including fishing and aquaculture, mowing and pruning from public and private green areas, as well as the biodegradable part of industrial waste and urban”.

Obviously, the cultivation of energy crops in soil used for arable crops (food and feed crops) has raised serious concerns, in particular to the competition that is regarding with the use of the soil and causing a reduction of the area destined to food crops to produce biomass crops and causing a change in the use of soil (Cosentino et al., 2014a).

With this objective, the European Union has issued several directives to promote the use of energy from renewable sources, to increase energy efficiency and reduce greenhouse gas emissions.

Subsequently, given the objectives of the European Union in the field of renewable energies and the lack of agricultural land to be used for the cultivation of non-food energy crops in different European regions, the problem of the destination of land use has caused significant repercussions on cultivated land with a high carbon content and has led to a change in the use of the land.

The cultivation of energy crops in marginal soils (soils that are not currently used and therefore destined for the cultivation of food crops) is possible in all those areas that present significant natural constraints (extreme climatic conditions, low soil productivity, steep slope, etc.).

The definition of marginal land is not univocal, because there could be different definitions differentiated according to the region in which they are located and to the time. First of all, it is essential to define the concept of soil and then classify the different types of soil present in Europe and Italy and in particular in the Mediterranean environment, in order to recognize marginal soils and be able to allocate them to a correct use such as the production of crops energy from biomass (Lewis and Kelly, 2014).

The soil

The soil is a natural body characterized by layers (horizons) and is composed of minerals, organic substance, air and water. It is the product of the influence of climate, topography, living organisms (flora and fauna) on the mother rock over time. It could be better defined as the result of a very long-lasting action, caused by the physical-mechanical action (alternation of high and low temperatures, frost, roots, wind), by the chemical action (water, CO_2, O_2), environmental factors and biological factors such as living organisms (terrestrial organisms, plant roots) on rocks and minerals on the earth’s surface, this alteration process is called pedogenesis[5]. Soil formation times are very long and for this reason the soil is considered a non-renewable resource (Bonciarelli, 1995).

In 2006 the European Commission published communication no. 231 “Strategies for soil protection” in which the soil has been defined as the top layer of the earth's crust, composed of minerals, organic matter, water, air and living organisms.

Soil performs fundamental functions for the life beings on earth because it provides us with food, biomass and raw materials; it represents the interface between earth, air and water and also a house for the biosphere.

It is a surface where all human activities take place, it is an element of the landscape, of the cultural heritage and plays a fundamental role as habitat and gene pool.

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[5] Pedogenesis (from the Greek πέδον, “soil” and γένεσις, “birth”) is the set of physical, chemical and biological processes that cause the soil formation over time, starting from the pedogenetic substrate, a rock material resulting from a first alteration of the rock mother (the original lithological material).

Water, nutrients and carbon are stored in the soil and are of great importance for the socio-economic-environmental environment.

The fragility of the soil ecosystem was questioned for the first time by E.H. Faulkner in 1943 in the writing “Folly of Plowman” where he describes the phenomena called "dust bowl" and reveals the great vulnerability of the plowed lands in the southern United States between 1931 and 1939, the years following the economic crisis of 1929 they also led to disastrous consequences for crops and entire farms.

This condition was mentioned in the memorable statement by the President of the United States F.D. Roosevelt in 1937 (… a nation that destroys its soil destroys itself) highlighting for the first time the concept of soil sustainability as a support for human activities and for the irreplaceable functions it performs (Schröder et al. 2018).

The soil term in the agronomic literature generally indicates the surface layer of the emerged lands where plants expand their root system, offers mechanical support and nourishment of water and mineral salts; it also includes a wide range of soils, from defined soils “in situ” (agricultural land) to substrates placed in pots, and although the two concepts are different the term soil is generally used like synonymous (Giardini, 2012).

Classification

As long as agriculture was a mainly empirical activity, linked to local conditions, little attention was paid to the description of the soils. They were defined with local terms that did not have an informative value for those who did not know local reality and local terminology.

In the first half of the XX^th^ Century, the study of agriculture was tackled on a scientific basis and this revealed the need to rationally define the fundamental environmental component that is soil and consequently in many countries a system of classification of soils.

The most important factor to determining the evolution of the pedogenetic process and therefore the making of the different soil types is the climate.

A first climatic classification of the soil is the Baldwin classification in 1938, this provides a very clear general picture and adopts a terminology that has found application in the subsequent classification of soils such as the French classification, the soil taxonomy of the United States and the soil classification of the FAO in 1974.

Certainly, soil classifications are an indispensable basis for carrying out technical intervention in a defined area, however it is difficult, for those are not specialists, to evaluate the potential use of land compared to another, for example to evaluate the agricultural suitability. Therefore, various soil classification systems have been proposed on their suitability for use[6].

These classification systems based on the intended use of the soils are important tools used to plan proper land use. There are several classification systems for the use of soils: Land capability USDA; Land suitability; Land classification for irrigation purpose; FAO soil productivity scale.

FAO (United Nations Food and Agriculture Organization) defines soil in its traditional meaning as the natural site for

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[6] IUSS Working Group WRB, 2015. International Soil Classification System for Naming Soils and Creating Legends for Soils Maps. World Soil Resources Reports No. 106, FAO.

plant growth. Soil is an essential component of the environment and ecosystems. For the functions that the soil performs, it provides humanity with the ecosystem services necessary for its support. Incorrect agricultural, zootechnical and forestry practices, settlement dynamics, changes in the destination of land use and local effects of global environmental changes cause serious degradation processes that limit or totally inhibit the functionality of the soil in an irreversible way. Other phenomena of soil degradation are the increase in artificial land cover, the construction of new buildings, sheds, settlements and the expansion of cities.

Soil functions fall into two main categories, ecological and socioeconomic. The first includes the functions of biomass production, protection of pollutants against the food and water chain, biological habitat and genetic reserve, fertility and quality. The second function includes support for civil and industrial settlements and recreational activities, valorization of effluents and residues from the treatment and disposal of waste, protection and conservation of historical and archaeological heritage.

Italian soil types

Italy has a great variability in terms of climatic, lithological, orographic conditions, etc. that causing the pedogenetic process. This meant that are several Italian soils. The first systematic study of Italian soils dates back to the creation of the 1: 1.000.000 scale Italian soil maps in 1966 and this classified soils according to the French classification. Below are the main types of Italian soils and their description, location and agricultural productivity.

Lithosols and regosols are young and not very evolved soils because the lying position effect erosion. The lithosols are developed on hard and compact rock, are found throughout the national territory and have very little agricultural potential. The regosols are formed on soft rocks and are found in the clay formations of the Sicilian hill and in a belt that runs along the Ionian Sea. Are clay soils, often subject to spectacular erosion like badlands and to landslide instability. Agricultural productivity is poor but with good technique it could be moderate even if only for cereal-zootechnical cultivation systems. The negative characteristic that gives rising a low productivity level in these soils is the poor physical state because the high clay and bad structure determines a bad penetration of both water and air and roots. Are ascribable to the order of Entisols in soil taxonomy.

Rankers and rendzina are soils at in an advanced stage of development, characterized by a shallow profile with a surface horizon rich in organic matter and present a landscape very rough. Rankers develop on siliceous rocks and are almost acidic and rich in skeleton; they are found in the Alps, in the Apennines but also in Calabria and Sicily. Their agricultural potential is poor for their small thickness and their use is limited to grazing or woodland.

Rendzina instead evolve on limestone rocks, are rich in skeleton and have a neutral or subalkaline reaction for the calcium carbonate, have strong erodibility if the natural plant surface is removed. In soil taxonomy rankers find their place in the order of Inceptisols, rendzina in the Mollisols.

Inceptisols are soils characteristic of volcanic areas where they evolve on lava and pyroclastic materials. They are found mainly on Etna, in the smaller islands that surround Sicily, on

Vesuvius, in Sardinia and in Lazio. They often have excellent agronomic characteristics: low density (they are soft), high exchange capacity, high water retention capacity and subacid reaction. Their agronomic potential is good unless it is limited by altitude or stony. In soil taxonomy they are placed in the order of Inceptisols (Yong and Warkentin, 1975).

Vertisols are soils of considerable thickness and uniformity, the main characteristic is the high percentage of expandable clay (30%-70%); the organic substance is not higher but well humified and strongly linked to inorganic colloids, so these soils have a good stable structure and characteristic dark color; the great water retention capacity and the reaction is subalkaline. Their agricultural potential is high, with a vocation for open field herbaceous crops, but also for vineyards and horticulture. They are widespread in western Sicily and other regions of southern Italy. The vertisols correspond to the homonymous order in the soil taxonomy.

Alluvial soils are present in all Italian plains and in the valley bottom. They are soils with a high heterogeneity of mineralogical composition of texture and for having been deeply reworked by man. These are soils with good production potential, especially if improved with systematic interventions that ensure drainage., Especially if improved with hydraulic operations that improve drainage. In the soil taxonomy they are ascribed to the order of Entisols.

Brown soils are a vast group of soils that present an alteration horizon and that have evolved on substrates of various lithological nature. They have a significant but not optimal thickness; the texture is basically free; the reaction varies from subacid to subalkaline; the limestone content is medium to high; the content of mineral elements is variable, improved by fertilizations. The typical natural vegetation is the deciduous forest, but their intended use is both herbaceous and arboreal crops, given that their production potential is good. For their remarkable heterogeneity, they have difficult to frame in the pedological classifications (Inceptsols, Mollisols) and must be distinguished on the basis of particular aspects (acids, limestone).

Acid brown soils have evolved on arenaceous substrates and under high rainfall climates. Their agricultural potential is discreet, enhanced with low-altitude tree crops. They are found in the Apennine areas and in the Alps. The brown calcareous soils have a high carbonate content, subalkaline reaction, not very deep profile, good supply of nutrients. They are widespread in Italy and their agricultural potential is not very high and their prevalent uses are grazing, arable crops and marginal arboriculture such as almond and olive trees. Red lands are formed on limestone rocks on the whole peninsula, and in particular in the southern and island regions. The red soils formed on limestone massifs stand out, with a steep slope with thin soil and outcropping rock and the flat red soils of the coastal planks present in Puglia and Sicily. The latter have a low agricultural potential, but it can be increased with agronomic interventions and with irrigation that makes possible valuable cultivation addresses (citrus orchards, orchards, vineyards, vegetable gardens). The red lands are placed in the Alfisols order of the soil taxonomy.

Podzols and podzolic soils (brown podzol) in Italy are land with various podzolization[7] degrees; they are found in high

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[7] The pedogenetic process of podzolization consists in the removal of organic and inorganic colloids from the surface layer which acquires the characteristic ash color and in their

mountain regions with a continental climate, on siliceous rocks, under conifers or ericaceous. The agricultural potential is very low given the strong acidity, the chemical poverty of the surface layer, the compactness of the river horizon. In soil taxonomy these soils fall into the Spodsols.

Saline soils (alomorphs) are found in the semi-arid environments of southern Italy. These soils refer to Entisols and Inceptisols.

EU Regulations

Substantial and innovative changes had to be made to obtain more results. The Renewable Energy Directive (RED) No. 2018/2001 has made several substantial and innovative changes compared to the previous EU Directive No. 2009/28[8] as regards both the use and the supply of energy starting from renewable resources by the member states of the European Union.

This new EU Directive established new sustainability and greenhouse gas emission reduction criteria for the production of biofuels, bioliquids and biomass fuels. According to what is issued in article 1, this EU Directive establishes common rules for all Member States to promote the production of energy from renewable sources.

The binding target for all EU member states establishes to reduce greenhouse gas emissions by at least 40% until 2030 compared to the estimated levels of the year 1990.

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transport at the bottom a brownish red horizon is formed due to the presence of humic compounds and sesquioxides.

[8] EU Directive No. 2009/28 of the European Parliament and Council of 23 April 2009 about the promotion of the use of energy from renewable sources, that repealing EU Directives No. 2001/77 and EU Directive No. 2003/30 (OJ L 140 of 5.6.2009, page 16).

For the European Union, the promotion of the correct and efficient use of all those forms of energy obtained from renewable resources represents one of the primary objectives of the European Union's energy policy in accordance with the provisions of Article 194 of the Treaty on functioning of the European Union (TFEU).

To reduce greenhouse gas emissions and comply with the obligations signed by the European Union in Paris 2015 during the 21ª Conference of the United Nations Convention on Climate Change, also called "Paris Agreement" or COP21, it is necessary for all member states to deal concretely with the production of energy obtained only from renewable sources "renewable energy" and this is the application of the package legislation that includes all EU energy and climate change policies.

Other elements are contained in this new EU Directive, such as the development of new fuels for transport obtained from renewable energy sources and the production of energy from renewable sources.

The European Commission defined on 22 January 2014 the "Framework for energy and climate policies from 2020 to 2030". The Commission proposed that the European Union could achieve a target of 27% renewable energy consumption by 2030 and a 32% renewable energy production by 2030.

The EU regulation no. 1099/2008, directives no. 2001/77, no. 2003/3 and no. 2009/28 defined the different types of energy that can be obtained from renewable sources. Among renewable energy sources, the availability of biomass must also be considered as a sustainable energy source in accordance with the provisions of EU directive no. 2008/98. Reducing waste production and promoting recycling of waste must be a priority for all Member States.

The EU directive no. 2009/28 introduced sustainability standards, also for soil protection especially for soils that have a high biodiversity and high carbon stocks. Unfortunately, it has failed to solve the problem of land use change; when, for example, biomass crops for energy are grown on land intended for the production of food and forage crops. All of this increased the pressure on the soil and shifted agronomic production to areas with high carbon reserves, such as forests, wetlands and peat bogs, causing an increase in greenhouse gas emissions.

The EU regulation no. 2018/1999[9] it includes several measures to promote energy efficiency in the Member States of the Union, to reduce greenhouse gas emissions and to eliminate energy dependence on third countries.

Necessary is to promote the use of raw materials obtained from biomass to obtain biofuels because they have a low environmental impact both as regards the change in the use of the land and for decarbonization. It is possible to mitigate the change in the destination of land use by promoting the cultivation of biomass crops for energy to obtain biofuels, bioliquids and fuels in those soils that have never been previously cultivated. In the new EU directive no. 2018/2001, biofuels, bioliquids and fuels obtained from biomass raw materials are considered low risk as regards the variation in the intended use of the land.

In this EU Directive, in particular in the Article No. 2, there are definitions that already come from the previous EU

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[9] EU Regulation No. 2018/1999 of the European Parliament and Council of 11 December 2018.

directive n.2009/72[10]:

- Energy from renewable sources or renewable energy is energy obtained from non-fossil renewable sources like wind, solar (solar thermal and photovoltaic), geothermal energy, environmental energy, wave motion and other forms of sea energy, hydraulic energy, biomass, landfill gas, residual gases obtained from the treatment process and biogas;
- Waste: defined in the Article No. 3 point 1 from EU Directive No. 2008/98[11] excluding substances that have been deliberately modified or contaminated;
- Biomass: the biodegradable fraction of products, wastes and residues of biological origin from agriculture, like plant and animal substances, forestry and related industries, including fishing and aquaculture, as well as the biodegradable part of the waste, including industrial and urban waste of biological origin;
- Agricultural biomass: biomass from agriculture activity;
- Forest biomass: biomass from forestry;
- Biomass fuels: solid and gaseous fuels obtained from biomass;
- Biogas: gaseous fuel produced from biomass;
- Organic waste: defined in the Article No. 3, point 4 of EU Directive No. 2008/98;

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[10] EU Directive No. 2009/72 of the European Parliament and Council of 13 July 2009 on common rules for the internal market in electricity and repealing EU Directive No. 2003/54 (OJ L 211, 14.8.2009, p. 55).

[11] EU Directive No. 2008/98 of the European Parliament and Council of 19 November 2008 on waste and repealing certain directives (OJ L 312, 22.11.2008, p. 3).

- Supply area: defined a geographical area where are from forest biomass raw materials;
- Forest regeneration: rebuilt with natural or artificial means of a wooded area after removing the forest population for felling or natural causes, including fires or storms;
- Bioliquids: liquid fuels for energy purposes including electricity, heating and cooling, produced from biomass;
- Biofuels: liquid fuels for transport derived from biomass;
- Advanced biofuels: biofuels produced by raw materials listed in Annex IX, Part A;
- Fuels derived from recycled carbon: liquid and gaseous fuels produced from liquid or solid waste non-renewable that are not suitable for recycling mentioned in EU Directive No. 2008/98 Article No. 4 or gas deriving from waste treatment non-renewable produced by industrial process;
- Renewable non-biological liquid and gaseous fuels for transport: liquid or gaseous fuels that are used for transport, that comes from renewable sources different from biomass;
- Biofuels, bioliquids and biomass fuels with low risk of changing soil use: biofuels, bioliquids and biomass fuels from raw materials have been produced in systems that avoid the effects of displacement of biofuels, bioliquids and biomass fuels obtained from food and fodder crops through the improvement of agricultural practices and in cultivation areas not previously used for this purpose, produced by sustainability standards for biofuels, bioliquids and biomass fuels from the Article No. 29:

- Starch crops: cereals, where is used grains and also the whole plant, for example the green corn; tubers and roots, such as potatoes, Topinambur, sweet potatoes, manioc and yams; and bulb-tuberous crops, such as Colocasia and Xantosoma;
- Food and fodder crops: starch, sugar or oil crops produced as the main crop on agricultural soil, excluding residues, waste or ligno-cellulosic materials and intermediate crops, such as catch crops and cover crops;
- Ligno-cellulosic materials: composed by lignin, cellulose and hemicellulose such as forests biomass, wood energy crops and forestry chain waste;
- Cellulose materials by non-food origins: raw materials composed by cellulose and hemicellulose with a lignin content lower than lignocellulosic materials, including waste by food and forage crops, such as straw, corn stalks, husks, low starch herbaceous energy crops, such as ryegrass, panic rod, miscanthus, giant reed, cover crops and subsequent crops, mixed legume and grass crops, industrial waste, including food and fodder crops waste like vegetable oils, sugars, starches and proteins, and materials derived by organic waste, mixed crops of legumes and grasses and mixed association of cover crops grasses and legumes with low starch content and cultivated to produce fodder for livestock and improved soil fertility to obtained higher yields from the main arable crops;
- Residue: substance different from the final products obtained by the production process;
- Residues from agriculture, aquaculture, fishing and

forestry: generated by agriculture, aquaculture, fishing and forestry practice they do not include residues from the industry process.

Article No. 3 of this EU Directive sets the binding target for Member States for 2030 which will have to produce energy from renewable sources for final consumption in a share equal to 32% according to paragraph 1 of this article and that Member States to achieve the binding objective, they must follow the procedure indicated in the Articles No. 9 and No. 31 of regulation 2018/1999. According to this article, from 1 January 2021, the share of energy from renewable sources in each Member State must not be less than the basic share indicated in the third column of the table in Annex I, Part A of this Directive. The European Commission has set up a platform to support Member States that use cooperation mechanisms to contribute to the European Union target set out in paragraph 1.

Article No. 19 discusses the guarantees of origin of energy from renewable sources. All these specific rules for biofuels, bioliquids and biomass fuels obtained from food and fodder crops are mentioned in the Article No. 26.

Furthermore, in the same article, the procedures for estimating the final consumption of energy from renewable sources obtained by a Member State referred to in the Article No. 7 and the minimum quota referred to in the first paragraph of the Article No. 25 are established. The share of biofuels and bioliquids, as well as biomass fuels for the transport produced from food or fodder crops, must not exceed 1% of the final energy consumption share in 2020, with a final energy consumption of up to 7% for the sectors road and rail transport in the Member States.

The standards of sustainability and reduction of greenhouse gas emissions for biofuels, bioliquids and biomass fuels are dealt with in the Article No. 29. Instead, article 30 verifies the standards of sustainability and reduction of greenhouse gas emissions. While all the rules on the impact rate of greenhouse gases, biofuels, bioliquids and biomass fuels are indicated in the Article No. 31 of this Directive.

Annex I deal with the general national targets for the share of energy from renewable sources in final energy consumption in 2020. Annex III contains the tables relating to the energy content of the fuel. Annex V contains the rules for calculating the greenhouse gas impact of biofuels, bioliquids and reference fossil fuels.

The issue of CO_2 it is not taken into consideration for the cultivation of raw materials and its extraction (including the emissions deriving from the extraction or cultivation process, collection, drying and conservation, waste and production and products used for their extraction and cultivation). The emission rates resulting from the cultivation of agricultural biomass are included in the Article No. 31 (4) or could be obtained with standard emission values obtained from the crops included in this annex, as an alternative to the use of the actual values. In the absence of relevant information, averages based on agricultural practices can be calculated using, for example, data derived from groups of farms, as an alternative to actual values. Annual emissions resulting from carbon stocks that change after changing land use could be calculated by evenly distributing total emissions for 20 years. This annex defines “heavily degraded soils”: those soils that have long been highly saline or the content of organic matter is particularly low and / or have undergone intense and prolonged erosion over time. Also defined as “waste and residues”, to include (fronds and tree branches, straw, peel, cobs and shells, processing residues, raw glycerine and bagasse) are considered materials with zero gas emissions, because they are transformed into intermediate products before being a finished product.

Annex VI contains the rules for calculating the greenhouse gas impact of biomass fuels and fossil fuels. The reduction of greenhouse gases for biomass fuels can be achieved if they have been produced without net carbon emissions following the changing use of soils. The emissions deriving from the extraction, collection or cultivation of raw materials include: the emissions deriving from the extraction, cultivation or collection process; from the collection, drying and conservation of raw materials; from waste and from the production of chemicals used in their extraction or cultivation. The capture of CO_2 it is not taken into consideration in the cultivation of raw materials. The estimate of the emissions derived from the cultivation of biomass could be derived from the reports included in the Article No. 31 of this directive or from this annex, as an alternative to the use of actual values. In the absence of relevant information in these reports, averages calculated with reference to agricultural practices based, for example, on data from farm groups, as an alternative to the use of real values, may be possible. In Annex IX there is a first part called “Part A” where there are all those raw materials used for the production of biogas, biofuels for transport and advanced biofuels, which give a double contribution compared to their energy content to achieve minimum quotas as defined in article 25. These raw materials are:

- Algae, growning on land in ponds or photobioreactors;

- Biomass fraction from municipal waste, as defined in to Article 11, paragraph 2, letter a, of EU Directive No. 2008/98;
- Organic waste as defined in Article 3, point 4 of EU Directive No. 2008/98, coming from domestic collection and subject to separate collection referred to in Article 3, point 11, of the same Directive;
- Biomass fraction corresponding to industrial waste not suitable for use in the human or animal food chain, including material from the retail and wholesale trade and from the agri-food, fishing and aquaculture industry, and excluding the listed raw materials in the so-called “Part B” of this annex;
- Straw;
- Animal fertilizer and sewage sludge;
- Effluent from oil mills that process palm oil and empty palm fruit bundles;
- Tall oil pitch;
- Raw glycerin;
- Bagasse;
- Pomace and lees of wine;
- Hulls;
- Corncobs cleaned of corn grains;
- Biomass fraction like waste and residues from the forestry industry and activity, namely bark, branches, pre-commercial thinning products, leaves, needles, crowns, sawdust, splinters, black lye, brown sewage, fiber sludge,

lignin and tall oil;

- Other cellulose materials of non-food origin;
- Other wood-cellulose materials, except saw logs and veneer logs.

"Part B" of this annex deals with the raw materials used for the production of biofuels and biogas exclusively and exclusively for transport. Their contribution to the achievement of the minimum quotas established in the first paragraph of Article No. 25 is limited and could be considered twice the energy content and are: used cooking oil; animal fats classified as categories 1 and 2 for the EU Regulation No. 1069/2009.

In Annex X "Part A", there are all the EU directives that have been abolished with this new directive and its amendments are present in Article No. 37.

iLUC Criteria

Following the application of the renewable energy directive adopted by the European Parliament[12], which has already entered into force, the Commission has also adopted a delegated act[13] which establishes the criteria for determining which iLUC high-risk raw materials are. The acronym "iLUC" indicates the production of crops from biofuels in production areas that have land with a high carbon

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[12] EU Directive No. 2018/2001 of the European Parliament and Council of 11 December 2018 on the promotion of the use of energy from renewable sources.

[13] EU Commission Delegated Regulation of 13.3.2019 supplementing to EU Directive No. 2018/2001 as regards the determination of high indirect land-use change-risk feedstock for which a significant expansion of the production area into land with high carbon stock is observed and the certification of low indirect land-use change-risk biofuels, bioliquids and biomass fuels.

content, therefore, land intended for food and forage crops. The criteria for determining whether an indirect change in land use has occurred to produce biomass crops to obtain biofuels, bioliquids and fuels are indicated in an annex[14].

Biofuels are defined as all those liquid fuels obtained from biomass and used mainly for transportation. The most important biofuels still used today are bioethanol (produced from crops such as sugar cane and cereals), which is used as a substitute for unleaded petrol and biodiesel (mainly produced from oil crops and therefore production of vegetable oils) which is considered replacement of diesel engine fuels. Bioliquids, on the other hand, are liquid fuels obtained from biomass and used mainly to produce electricity, for heating and / or cooling systems. Biomass fuels are solid or gaseous fuels obtained from biomass crops. Therefore, all these fuels are made with biomass crops. They have different names depending on their physical nature (solid, gaseous or liquid) and their use (for transportation or to produce electricity, heating and / or cooling).

The acronym iLUC indicates all those lands previously used as pastures or for agricultural production destined for the production of food and / or feed which have been converted and used for the production of biomass crops to subsequently obtain biofuels. With the problem of an increasing increase in the demand for food and feed crops, these crops are therefore destined for soils that are located in areas with a high carbon stock such as forests, wetlands and peat bogs. This causes a change in land use (transforming these “virgin”

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[14] Supplementing EU Directive No. 2018/2001 as regards the determination of high indirect land-use change-risk feedstock for which a significant expansion of the production area into land with high carbon stock is observed and the certification of low indirect land-use change-risk biofuels, bioliquids and biomass fuels

areas never used for agricultural production into agricultural land). This leads to an increase in greenhouse gas emissions (CO2 that was previously stored in trees in forests and soil).

Unfortunately, this is at odds with the EU's main goal of increasing the use of biofuels instead of fossil fuels to reduce greenhouse gas emissions (CO_2). The EU directive provides for two different measures to deal with the iLUC problem.

The EU Directive sets national limits for biofuels, bioliquids and fuels obtained from biomass produced from food crops or feed, since these fuels have a high iLUC risk.

The EU directive has established national limits for each member state for 2019 and for the period 2021–2023 which, after 31 December 2023, will gradually decrease to reach zero by 2030, for biofuels, bioliquids and biomass deriving from high-risk crops, therefore those fuels produced from food or feed crops produced in those soils with a high carbon stock which are called iLUC (high risk fuels). The EU Directive introduces an exemption from these limits for all biofuels, bioliquids and fuels obtained from low risk iLUC certified biomass crops. iLUC high risk fuels are all those fuels obtained from food or feed crops that are grown in those soils with a high carbon stock such as forests, wetlands and peat bogs. This transformation in use from “virgin” soil (land that has never been cultivated) to cultivated land causes the release of a significant amount of greenhouse gas emissions (CO_2) and therefore is in stark contrast to the main objective of the EU directive namely, to reduce emissions from the use of fossil fuels, for this reason they cannot be considered renewable energy sources. There are no limits on the import or use of these fuels. Member States will still be able to import and use fuels included in the iLUC high risk biofuel category. The limitation established by the EU iLUC High Risk Fuel Directive only concerns the quantity of these fuels in the global national share of renewable energies and in the share of renewable energies used in the transport sector. Member States will have to reduce the percentage of iLUC high risk fuels to zero as a percentage to achieve the objectives set by the EU Directive. The EU Renewable Energy Directive introduces a new approach to addressing indirect emissions resulting from land use change (iLUC) associated with the production of biofuels, bioliquids and biomass fuels. The directive introduces an exemption from these limits for biofuels, bioliquids and biomass fuels certified as low risk iLUC. This delegated act establishes criteria for: determining iLUC high-risk raw materials and how to certify iLUC low-risk biofuels, bioliquids and biomass fuels. iLUC high-risk fuels are produced with raw materials in soils with a high carbon content.

The global production area for biomass crops has increased by more than 1% every year and 100.000 hectares after 2008. Biomass crops that have improved production without expanding the production area do not generate very high levels of greenhouse gas emissions because areas for their cultivation have not been deforested (CO_2).

Over 10% of this expansion of the cultivation of biomass crops has taken place on land with a high carbon stock. These data are included in the annex of the DG ENER report[15]. Biofuels, bioliquids and low-risk iLUC biomass fuels (low-risk iLUC fuels), defined in the EU Renewable Energy Directive, are all those fuels produced to mitigate iLUC emissions because they are the result of an increase in the

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[15] https://ec.europa.eu/energy/en/topics/renewable-energy/biofuels/sustainability-criteria

productivity of food crops or because they come from crops grown in abandoned or severely degraded so-called “marginal soils”. In accordance with the sustainability criteria established in the EU Renewable Energy Directive, which implies that raw materials can only be grown on marginal land not used for the cultivation of food or agricultural feed and which are not rich in carbon stocks. Compliance with these criteria can be verified through voluntary systems that have been recognized as valid by the Commission. These voluntary schemes[16] have already been used in the certification of the sustainability criteria established in the EU Renewable Energy Directive currently applicable for biofuels and bioliquids. It is an improvement of the global environmental benefits of European biofuels policy. Having established clears for the certification of low-risk fuels iLUC will also provide incentives to increase productivity especially in the agricultural sector. These targets will reduce pressure on forests and all soils with a high carbon content.

The EU Regulation No. 1305/2013 plans to outline the so-called “Areas with specific constraints”. This regulation through the combination of biophysical criteria in Annex III specifies that when at least two biophysical criteria are present within a margin that does not exceed 20% of the initially defined value, these agricultural areas could be defined as “Areas with specific constraints”. A group of ad hoc experts under the leadership of the JRC[17], has prepared the criteria and recommendations for a correct combination

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[16] https://ec.europa.eu/energy/en/topics/renewable-energy/biofuels/voluntary-schemes

[17] Joint Research Centre, Institute for Environment and Sustainability (Ispra) of the European Commission.

of the criteria and related thresholds present in the aforementioned EU Regulation. However, experts also pointed out that these criteria present uncertainties due to the lack of data availability and the complexity of the interactions existing between soil-environment-plant. The eight biophysical criteria (14 subcriteria) were crossed among themselves to obtain 91 pairs of possible purchases. These can be negative or positive interactions, or not giving interaction or giving unclear interactions. An assessment of the threshold values was made within the 20% margin and this revealed that the application of the threshold value is not always applicable. The scientific recommendations contained in the report leave the EU Regulation unchanged no. 1305/2013.

This report intends to provide recommendations on how to outline all those “Areas with specific constraints” based on the provisions of article 32.4 of EU Regulation no. 1305/2013. It explains how to combine the two criteria listed in Annex III of the regulation, within a margin of 20% of the threshold value. The report is divided into three main sections, immediately a brief description of the revision of the “Areas with natural constraints” present in the European territory, therefore the "Areas with specific constraints" are taken into consideration. The evaluation of combinations of criteria pairs is performed through a table in a cross section. This led to the identification of six different situations, three of which were relevant for outlining the “Areas with specific constraints”. Support for agriculture in mountain areas or other areas subject to natural constraints was aimed at compensating farmers because their agricultural production was exposed to adverse biophysical conditions related to soil, the environment and the slope of the soils. There are other supports necessary for proper land management to save or improve the environment, maintain agricultural activities in these lands, preserve tourism or protect the coast. These areas have been called "least favored areas" (LFA) in the past and support has been approved to prevent land desertification and biodiversity loss. Areas subject to natural or specific constraints other than mountain areas are subject to other requirements. The three categories defined "mountain areas", "Areas subject to natural constraints" and "Areas with specific constraints" (ANC) are regulated for the period 2014–2020. But the novelty introduced in the new EU regulation required the obligation for EU Member States to define the so-called "Areas subject to natural constraints" and the possibility of outlining the "Areas with specific constraints". EU Member States must review the criteria for the designation of "Areas subject to natural constraints" other than mountain areas and, if applicable, with specific constraints.

New criteria have been adopted by EU regulation no. 1305/2013 of the European Parliament and of the Council of 17 December 2013 on support for rural development by the European Fund for Agricultural Development of Rural Areas (EAFRD). The methodology for the designation of these areas is defined in Article 32, while the criteria to be adopted are listed in Annex III of the same regulation. The set of eight biophysical criteria and critical thresholds could be used for designation areas, other than mountains, with natural constraints. This has been defined by experts in the evaluation of the territory. The application of the methodology is based on Liebig's minimum agronomic law. These indicators could be used as criteria for classifying land in the EU-28 into 2 main classes:

1. soils without significant constraints and / or environmental problems and / or without slope;
2. soils with serious and / or environmental and / or slope problems, limiting agricultural activities.

The constraints were considered on the basis of the conventional European mechanized agricultural unit that produces cereals or haymaking for cattle breeding. This set of indicators could also be extended to the so-called "problematic soils" indicated by FAO for agricultural activities, while the threshold values are derived from scientific knowledge and consultation of experts. Biophysical data sheets are also described in Van Orshoven et al. 2014.

In the initial evaluation scheme of the territory to outline areas with significant natural constraints (ANC) for agriculture other than mountain areas, individual criteria were applied according to the Liebig minimum law. This approach would have defined that, at the threshold level, each criterion has a different influence on the suitability of the land and could present constraints on agricultural production. In this configuration, the criteria act independently of each other (Van Orshoven et al., 2014).

The legislator could be included the possibility of combining individual biophysical criteria in a margin of 20% from the initial value to identify the areas affected by specific constraints relating to cultivation activities. While biology recognizes the complexity of nature and agricultural land (the interactions between soil and environment and their impact on agricultural production). Unfortunately, environmental interactions may not be qualified, quantified and evaluated against a fixed value. Agricultural production does not respond linearly to these interactions. This type of evaluation has a greater degree of uncertainty and only specific data could be estimated from the site concerned and often through an approach based on cultivation processes. These data are not available semantically and geographically on a European scale and often not even at national or regional level. Given the specific sectors (agro-meteorology, crop growth, crop physiology, soil science) and many situations (from the combination of eight criteria), a group of experts from DG AGRI developed a methodology for the delimitation of "Areas with specific constraints".

The result of this analysis for a value of 20% or less has been described for each biophysical criterion and are: low temperature, dryness, excess soil moisture, limited soil drainage, poor structure and stonyness, reduced rooting depth, poor chemical properties, steepness and slope. The ANC criteria and values were taken from the EU regulation no. 2013/1305, but the different combinations of criteria were based on the pairs that could have led to a negative synergy by analyzing the group of experts. For 19 combinations, both criteria are not present in the same position; 21 combinations were not considered possible because it was not possible to define the threshold for one of the criteria; 18 combinations have no interaction; 5 combinations have been labeled with "unclear synergy". Only for combinations with negative synergy, it could be suggested to consider the area that presents a serious natural limit for agricultural activity when strict value limits have been indicated.

For all other cases, it has been proposed to use the Liebig minimum law, the criteria could be applied individually to the value indicated in Annex III of the EU regulation. The expert group developed combinations with negative, positive or unclear synergies. The information sheets for combinations of distinct pairs for negative, positive and unclear synergies are explained below only for those types of soil that affect the scientific experiment of the thesis work.

Negative synergy occurs when the combination of two sub-serious thresholds (below the serious thresholds) results in a combined and severe limitation.

Positive synergies are when the interactions determine a positive effect if two factors are combined together it is present a positive synergy.

Unclear synergies occur when both positive and negative synergies are detected and / or when the result of the combination depends on external factors that are not known or that can act differently from specific situation encountered.

Dryness × salinity

Soil salinity is one of the main environmental limits for agriculture worldwide, in particular in arid and semi-arid areas where the dry climate aggravates the negative effects of saline stress on crop productivity. The resulting stress can significantly limit the suitability of the soil for use as agricultural soil even in regions where climatic conditions would be suitable for agriculture (Pitman et al., 2002). Furthermore, surface evaporation caused by soil dryness could result in a high salt load on the soil surface, which can significantly increase the negative effect of salinity on crop emergence and on the growth of young seedlings (Abrol et al., 1988). The negative interaction between salinity and dryness can influence the plant-soil system in various ways. In saline soils, the low osmotic potential of the soil solution increases the minimum resistance that must be overcome to extract water from the pores of the soil. Prolonged drought conditions and high salinity levels also lead to ionic imbalances and deficiencies in the absorption of nutrients by plants, due to the effect of toxic ions on the absorption of nutrients from the solution circulating in the substrate. The main effect of salinity is the reduction of water absorption by plants which causes an increase in humidity even when water is still present in the rhizosphere (Munns, 2002; Taiz and Zeiger, 2009). In drought conditions, the loss of water caused by a strong evapotranspiration produces an increase in the concentration of salt in the solution of the circulating soil, with a consequent negative potential of the soil water. This causes a low transpiration rate, affects the transport of active ions and the permeability of the membrane, leading to a reduced absorption of nutrients and transport from the roots to the shoots. In this context, salinity also contributes to altering the nutritional status of plants, limiting the absorption of Ca^{2+}, K^{+} e Mg^{2+} ions through both physiological inactivation and competition effects, which in turn reduce the ability of plants to selectively absorb a specific nutrient in case of high concentration of Na^{+} e Cl^{-} in the flow soil solution (Taiz and Zeiger, 2006).

Stony sand × salinity

In sandy soils, crop growth may be limited for water stress and poor ability to retain water in the soil. Sandy soils have very little nutritional or supply capacity, therefore normal fertilization practices have limited efficacy. In addition, sandy soils are highly sensitive to erosion caused by water and wind and consequently require special soil conservation practices. Many cultivated and pasture plants cannot survive in high salt conditions. In saline areas with agricultural production, salinity has three main negative impacts on crops that can cause significant productivity losses. With increasing salinity, the assimilation of water by the soil by plants becomes more difficult. When the structure of the soil is damaged the growth of plants can be limited by the growing content of toxic substances such as dissolved salts (for example NaCl). The damaged soil structure and the reduced vegetation cover increase the risk of soil erosion caused by wind and rain (Van Orshoven et al., 2014)

The field capacity (the maximum amount of water that can be retained by the soil) is low in sandy soils. The quantity of water available is generally less than in soils with a fine texture and particularly reduced in medium and coarse sands. Water stress also occurs in sandy soils when the rains stop and a period of drought begins (Hall et al., 1977). Plant growth occurs as a function of total water stress (the sum of soil moisture and osmotic pressure of the solution circulating in the soil), this means that with increasing salinity in the soil, plants absorb water with increasing difficulty, increasing water stress. The level of saline groundwater increases and reaches the soil surface more quickly in sandy soils than in soils with a finer texture (Regional Salinity Laboratory, 1954). The salinity effect also manifests itself with a high risk of erosion of sandy soils. In fact, sandy soils are particularly sensitive to wind erosion due to their single grain structure (absence of aggregates) and the low amount of clay and humus (Shao, 2008). Since plant growth is reduced by salinity, the risk of erosion increases accordingly (Wolfe and Nickling, 1993). The tolerable limit of percentage of sand in a soil is 40%. While the tolerable salinity level is about ≤ 3.2 dS m^{-1} detected in the soil.

Stony and heavy clay × salinity

The combination of heavy clay and salinity in the surface soil leads to mutual interaction through the effects of salt and clay on soil moisture, nutrient availability and soil structure. As far as agricultural activities are concerned, the consequences of salinity are as different as the significant productivity losses with increasing soil salinity because it becomes increasingly difficult for plants to extract water from the soil (aggravation of water stress and drought due to the roots of plants) (Van Orshoven et al., 2014). In addition, the damaged soil structure reduces hydraulic conductivity and waterproofs the clay soil. The high salinity favors the structural stability of the clay, but the humidification of the soil reduces the concentration of salt and favors the formation of mud. An imbalance in the nutrient content occurs making them less available or toxic by limiting plant growth (Driessen et al., 2001).

In heavy clay soils, the salt may not be visible, as the salt crystals hide in the structure of the clay itself. With the rains, the salts can cause a peptization of the clay and the structured clay aggregates can turn into mud which, once dried, becomes a hard crust. The evaporation of stagnant water can release significant quantities of salts onto the soil surface. In addition, the presence of salt during the humid winters makes the clay soils muddy and waterproof. In saline clay soils the osmotic potential of the aqueous solution of the soil is added to the potential of the matrix (measured in kPa) to obtain a very high total water potential. The soluble salts move from the surface towards the depth, from relatively wet to dry areas, from irrigated fields to adjacent rainy fields, etc. Salts can also accumulate in areas with limited natural drainage (roads or railways). The negative synergy for the combination of the two criteria is that of soil with heavy clay and salinity is due to the fact that they cause an increase in drought stress. The constraint is then accumulated by the two factors. Threshold values can be set to the maximum allowed for both criteria when they occur in combination. It means areas with soils with a salinity between 3.2 and 4.0 dS m^{-1} and a clay content between 50 and 60%. The non-severe threshold for a soil with heavy clay is equal to a clay content of $\leq 50\%$. The non-serious threshold for salinity is ≤ 3.2 dS m^{-1} in the soil. The presence of saline soils occurs in river deltas and in coastal and river plains. Heavy clay soils are mainly found in river marshes and in marine and lake plains. If the capillary rise from shallow groundwater reaches the surface of the soil, salt accumulates on the surface (external salinization). This accumulation of salt from groundwater is typical in arid and semi-arid climates. The salt can also be brought by floods with sea water or lightly salted river water or by runoff from the surrounding sloping soils (Driessen et al., 2001).

Low rooting depth × salinity

Shallow soils can significantly limit crop productivity due to the reduction in the volume of soil in which cultivated plants can absorb water and nutrients. Furthermore, the presence of salinity in the rhizosphere can seriously compromise the economic and environmental sustainability of agricultural production. The problems depend on quantity and distribution of precipitation, substrate fertility, drainage capacity of so dense and shallow soils.

Furthermore, restrictions on the percolation of water at depth due to surface rocks prevent the leaching of salts beyond the rhizosphere, significantly limiting the possibility of reclaiming the soil. Salinity reduces the soil's ability to supply water to plants. Indeed, a high concentration of salts in the rhizosphere reduces the osmotic potential of the solution circulating in the soil, which in turn reduces the soil's water potential. As a result, the force that holds water in the soil increases, thereby reducing the amount of water available to plants (Munns, 2002; Castillo et al., 2007). This phenomenon is amplified in shallow soils. In addition, saline soils are prone to cracking and cracking due to the leaching of water further reducing the already scarce availability of water for plants grown in shallow soils. Salinity also has a negative impact on the nutritional status of the plant as it reduces the osmotic potential in the circulating soil solution thus limiting the intake of nutrients and causes a competitive effect between plants by reducing the selective absorption of K^+, Ca^{2+} e NO^{3-} when toxic ions (Cl^- and Na^+) are present in the circulating soil solution (Taiz and Zeiger, 2006). The minimum depth limit threshold for a soil to allow the development of the root system of plants is about ≤ 35 cm. The non-serious threshold for salinity in the soil is approximately ≥ 3.2 dS m^{-1}.

Salinity × excess sodium

Many cultivated and haymaking plants fail to survive high salt conditions. Salinity has three main negative impacts on crops which result in significant productivity losses (Van Orshoven et al., 2014). With the increase of salinity, the absorption of water from the soil by the root system of plants is very difficult, the soil structure is completely damaged and for the high content of toxic substances, the plants are not able to grow and produce; moreover, erosive phenomena occur mainly caused by wind and water because the soil has been completely altered in its structure and appears bare, that is, without vegetation. Sodic soils have two main negative impacts on agricultural production, both indirect in fact, the excess of sodium increases the risk of floods and the risk of erosion because it changes the physical properties of the soil (Tanji, 1990). Further negative impacts of sodium soils are the reduced availability of water for plants, the poor workability of the soil and sometimes the formation of a black crust on the surface consisting of dispersed organic substance (McCauley and Jones, 2005). The productivity of European sodium soils is lower than that of saline soils. Coexistence in a soil of salinity and sodicity leads to unfavorable conditions for the growth and development of most crops, therefore this coexistence causes absence of productivity. The saline soils of sodium become impermeable to water infiltrations (Qadir et al., 1998). The management of salinity and excess sodium is very complex when both conditions occur (NDSU Extension Service). Conventional remediation procedures that use gypsum for soil correction followed by leaching lead to poor economic returns. Excess sodium in the soil is often associated with soil salinity because Na^+ it is preferably absorbed with respect to Ca^{2+} and to Mg^{2+} with increasing salinity and due to the selective precipitation of calcium minerals when the soil solution undergoes evaporation phenomena (Bresler et al., 1982). The ratio between salinity (CE) and excess sodium (ESP) is the driving factor that determines the effects of salts and sodium on soils. The combination of salinity and excess sodium (saline-sodium soils) means that the concentration of water-soluble salt, the quality and distribution of the salts are similar to those of saline soils. There is also a massive layer with a columnar structure, which is characteristic of sodium soils (sodium subsoil). This columnar layer is usually located near the soil surface and at the same time it is the layer with the highest salt accumulation. Water management of these soils is impossible due to the very low water permeability caused by both the water-soluble Na salts and the exchangeable sodium content. The soil surface and sodium subsoil become extremely plastic when wet. Although salinity can somehow compensate for the effects of excess sodium that causes flocculation (Shainberg and Letey, 1984), has a lethal effect because it causes anoxia (Bresler et al., 1982; Suarez et al., 1984). The increase in the salt content reduces the productivity of the soil, therefore sodium soils with a higher salt content (salinity) are not productive and are not suitable for the development of agricultural crops. Natural vegetation is very poor, which is also proof of the very low productivity of saline-sodium soils (Borhidi, 2007) with an increase in the surface of bare soil without vegetation cover (Cisneros et al., 1999; Wang et al., 2013). The coexistence of salinity and excess sodium represent a strongly negative cohesion because it prevents the development of agricultural activities in these lands. The sub-severe threshold for salinity is ≥ 3.2 dS m^{-1} in the soil explored by the root system of plants. The sub-severe threshold for excess sodium is ≥ 4.8 ESP of the soil layer explored by the root system of plants.

Biomass crops suitable to marginal lands

The term “biomass” derives from the ancient Greek and refers to any biodegradable organic material from plants, animals and microorganisms, that is, from a living being. our goal is to observe and study the behavior of biomass from plants. Vegetable biomass is that organic material produced by any plant through chlorophyll photosynthesis, a biochemical process that starting from water and Carbon Dioxide present in the air and thanks to the sun energy, carbohydrates and chemical energy are obtained by plants. The biomass used to heat up or to cook food is the oldest renewable energy source, dating back to a million years ago, discovered by our ancestor Homo erectus during the Stone Age. In Europe, these traces have been evident for about 400.000 years (Gowlett, 2016).

Marginal lands also called unused lands in Mediterranean environment is affected by slope, salinity conditions and severe drought. From an agronomic point of view Perennial crops might have greater tolerance when grown in these soils because they require a lower demand for nutrient input and limited soil management than annual crops. Perennial grasses are C4 photosynthetic pathway and are more efficient than C3 plants in the use of abiotic resources like water (Fernando et al., 2010; Zagada Lizarazu et al., 2010).

In the Mediterranean environment the low water availability and the high temperatures during summertime indicated Perennial species like most promising species for energy and cellulose production. The Perennial species produced considerable amounts of lignocellulosic biomass from research carried out in recent years. Also, Perennial crops species are native, naturalized or have good adaptation capacity in these Mediterranean environment (Cosentino et al., 2005, 2007, 2008; Lewandowski et al., 2000).

Perennial grasses are indicated energy crops because have yield significantly more energy accumulated and used in their production than is required to their grow (Lewandowski and Schmidt, 2006).

According to FAO (2005), marginal lands are among the largest habitat types in the world. Their area is estimated at 52.5 million km^2, or 40.5% of the Earth's surface (European Commission, 2008).

According to EUROSTAT statistics, marginal lands occupied 70.5 million hectares in the EU-28 in 2013. This area represents 13% of the total area and 33% of the agricultural area used. The marginal lands are dedicated to the production of fodder for harvesting by grazing / peeling, cutting or both, or could be used for other agricultural purposes such as the production of biomass crops (Peeters et al., 2014).

Conservation of marginal lands also called grasslands is important to preserving biodiversity, to reducing CO_2 in the atmosphere, to preventing the risk of fires, to promoting recreational activities and tourism (Carrillo et al., 2014).

There is a whole range of perennial crops for biomass production which have been tested and selected as the most suitable for European marginal lands (Lewandowski et al., 2003; Zegada-Lizarazu et al., 2010; Cosentino et al., 2012).

Lignocellulosic perennial grasses

The term lignocellulosic refers to plant dry matter (biomass), so called lignocellulosic biomass. It is the most abundantly available raw material on the Earth for the production of biofuels, mainly bioethanol. It is composed of carbohydrate polymers (cellulose, hemicellulose), and an aromatic polymer (lignin).

These carbohydrate polymers contain different sugar monomers (six and five carbon sugars) and they are tightly bound to lignin. Lignocellulosic biomass can be broadly classified into virgin biomass, waste biomass and energy crops.

Virgin biomass includes all naturally occurring terrestrial plants such as trees, bushes and grass.

Waste biomass is produced as a low value byproduct of various industrial sectors such as agriculture and forestry.

Energy crops are crops with high yield of lignocellulosic biomass produced to serve as a raw material for production of second-generation biofuel. Many crops are of interest for their ability to provide high yields of biomass and can be harvested multiple times each year and these are called Perennial grasses classified like virgin biomass.

Perennial grasses are crops that are highly resource-efficient, efficient use of solar energy, water and nutrients present in the solution circulating in the soil and do not require further input (Kiniry et al., 1999; Cosentino et al., 2007a, 2014, 2016; Ceotto et al., 2013; Triana et al., 2014).

They adapt very well to growing on poorly, drained and flooded soils (Lewandowski et al., 2003; Mann et al., 2013), in presence of soil salinity (Sánchez et al., 2015; Anderson et al., 2015; Stavridou et al., 2016), on contaminated soil (Barbosa et al., 2015) and on steep slopes (Cosentino et al., 2015a). At the growing season they could present competition problem with weeds (Lewandowski et al., 2003; Zegada-Lizarazu et al., 2010; Scordia et al., 2015).

The main problem with perennial grasses is that they are still found only in the wild, although several studies have shown that present some genetic variability. Some perennial grasses are even unable to produce viable seeds resulting in limited genetic diversity such as A. donax L. and the Miscanthus × giganteus hybrid (Lewandowski et al., 2003; Zegada-Lizarazu et al., 2012, 2013; Berti and Johnson, 2013).

It is of primary importance to be able to establish a low costs protocol for breeding programs in order to obtain the certified seed (Ideotypes) and optimized to be cultivated in the various environmental conditions present on European areas. This will ensure that they reach their potential yield in a given environmental condition. The research is therefore focused on finding new genetic resources from wild germplasm and obviously on the study of physiological and productive characteristics.

So different species are more suited to different types of marginal land, and to different types of conversion process. Lignocellulosic crops generally have a higher GHG efficiency than annual crops since they have lower input requirements and the energy yield per hectare is much higher.

Arundo donax L.

Arundo donax L. is a potentially high-yielding non-food crop that meets the EU market requirements for energy and advanced biofuels, paper and pulp and construction materials. Contrary to Miscanthus, the Arundo donax L. has the advantage of being native to southern Europe. In some environments it was considered an invasive species and as such was subject to eradication.

The new market for biomass and in particular advanced biofuels for transport (road, air, maritime) and for other industrial products has determined a growing interest in this crop and its potential. The reduction of costs to produce biomass for energy could be obtained by increasing the crops through genetic improvement and the application of effective cultivation techniques.

In addition, the regulatory package for climate and energy requires significant reductions in greenhouse gases (GHG) in transport: 20% in 2030 and 60% in 2050.

The cultivation of energy crops including Arundo donax L., the most promising among the raw materials for energy, must be carried out in a sustainable way, therefore the cultivation on marginal land to do not subtract land from agricultural food production. Giant reed has several attractive characteristics that could make it the king of biomass crops[18].

Arundo donax L. is a perennial rhizomatous grass belonging to the Gramineae family (Poaceae) (Rossa et al., 1998; Lewandowski et al., 2003) and carries out the photosynthetic process like all C3 cycle plants but in a slightly different way more efficient.

Originating in Asia, subsequently it spreads to several subtropical wetlands and warm temperature regions in Europe, Africa, North America and Oceania.

A wide range of biomass crop yields is reported in the literature and depending on the production site, the climate, the soil and its fertility, the inputs, the cultivation and harvesting practices and the plantation age.

The Arundo donax L. has a high photosynthetic capacity which is different from other C3 species, in fact it is very similar to the C4 species. It is quite clear that the Arundo

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donax L. has a high photosynthetic capacity that is uncommon compared to other C3 species, in fact, it is very similar to that of the C4 species.

This high photosynthetic capacity is related to the absence of saturation in the absorption of CO_2 and in the transport of electrons through the photosystem II (Rossa et al., 1998).

The photosynthetic capacity of Arundo donax L. in full sunlight is high compared to other C3 species, and comparable to C4 bioenergy grasses however, it is still more efficient than most C3 species (Webster et al., 2016).

However, as a C3 crop, Arundo donax L. shows a high rate of leaf transpiration. In the field, under unlimited soil water availability, the transpiration rate reached 7,5 mmol H_2O m^{-2} s^{-1}, much higher than many C4 grasses. Thus, is able to achieve its high photosynthetic rates with substantial transpiration (Cosentino et al., 2016).

Origin

According to numerous phylogenetic studies, five species of Arundo donax L. have been identified in subtropical Eurasia (Hardion et al., 2012, 2014a, b), four of which in the Mediterranean area (A. donax L., Arundo micrantha Lam., Arundo plinii and Arundo donaciformis (Loisel)).

The origin of Arundo donax L. could be the result from: crossing between A. plinni and a diploid of the same species, with consequent sterile triploid, or crossing between a fertile tetraploid of A plinii and Phragmites australis, resulting in a sterile hybrid based on the number of chromosomes that is very high (110), although the origin of Arundo donax L. is still uncertain today (Bucci et al., 2013; Mariani et al., 2010;

Christopher and Abraham, 1971; Pizzolongo, 1962).

The existence of many varieties of this species has been known since the mid-1900s (Perdue, 1958). The area of origin of *Arundo donax* L., according to many authors, is located in eastern Asia (Polunin and Huxley, 1987; Fornell, 1990).

From an analysis of samples collected from 80 different sites, it suggested that this species had originated in Asia and later spread to various subtropical wetlands and warm temperature regions of Europe, Africa, North America and Oceania (Mariani et al., 2010).

Nowadays, numerous studies consider Italy to be naturalized habitats (Angelini et al., 2009; Cosentino et al., 2006, 2014; Mantineo et al., 2009; Mariani et al., 2010; Borin et al., 2013; Haworth et al., 2016), Spain (Sánchez et al., 2015, 2016a,b), Greece (Christou et al., 2003), the United States (Di Tomaso and Healey, 2003; Herrera and Dudley , 2003; Khudamrongsawat et al., 2004; Ahmad et al., 2008; Balogh et al., 2012; Minogue and Wright, 2016; Wunderlin et al., 2017), South Africa (Rossa et al., 1998), Egypt (Galal and Shehata, 2016) and Australia (Williams et al., 2008).

Although Arundo donax L. produces flowers, they are not viable seeds (Boose and Holt, 1999; Dudley, 2000; Spencer et al., 2005; Williams et al., 2009; Mariani et al., 2010; Balogh et al., 2012).

Consequently, its propagation and diffusion are carried out mainly by extension of the rhizome, fragmentation of the rhizome or by the effect of floods (Boose and Holt, 1999; Lewandowski et al., 2003; Boland, 2006; Mariani et al., 2010; Ceotto and Di Candilo, 2010; Saltonstall et al., 2010; Pilu et al., 2013).

Due to vegetative reproduction, low genetic variability has been observed among A. donax L. plants (Lewandowski et al., 2003; Khudamrongsawat et al., 2004; Ahmad et al., 2008; Touchell et al., 2016).

Its rapid growth rate and its easy propagation (Herrera and Dudley, 2003) together with its tolerance towards unfavorable environments and sterile soils have made *Arundo donax* L. the most common perennial grass in many environments thanks to its ability to naturalization and adaptation in areas where it was not an autochthonous species (Barney and Di Tomaso, 2008; Barney et al., 2009).

However, its non-viable seeds and its use in marginal soils are not obstacles to the use of A donax L. as an energy crop for biomass production while has being considered an invasive crop (Pilu et al., 2012).

Physiology

Biomass productivity is determined by calculating the net increase in the dry matter of the plant unit of light intercepted RUE (efficiency of the use of radiation), of breathable water WUE (efficiency of the use of water) or by absorption of nutrients NUE (nutrient use efficiency) (Kiniry et al., 2011).

The giant cane that grows in hot environments at temperatures and increasing solar radiation shows a rapid closure of the stomata and a growth rate, which allows this crop to intercept almost all the photosynthetically active radiation (PAR) available when the leaf area index (LAI) is greater than 4.0.

This occurs in the first 2-3 months after the spring growth according to research conducted in the semi-arid areas of the

Mediterranean (Cosentino et al., 2014).

In a field in northern Italy during the summer, the RUE values of the giant reed and *Miscanthus* x *giganteus* has been compared, with solar radiation, temperature and availability of water not limiting. The calculated RUE values were 2,02 g MJ^{-1} for giant barrel and 2,70 MJ^{-1} for *Miscanthus* x *giganteus*, in line with the RUE values of C3 and C4 species (Nassi or Di Nasso et al., 2011a).

In a field trial in southern Italy the giant reed was compared with different levels of nitrogen fertilization and different levels of water availability in the soil. The giant cane has been found to increase its RUE in proportion to the increase in nitrogen and available water. RUE values ranged from 1.26 g MJ^{-1} in rainy conditions to 1.94 g MJ^{-1} when 120 kg of N ha^{-1} yr^{-1} and well-watered were applied (Cosentino et al., 2016).

These RUE values were lower than those of Miscanthus x giganteus grown in well-watered conditions, 2.33 g MJ^{-1}, but higher than those in rainy conditions in the same experimental area (1.24 g MJ^{-1}) (Cosentino et al., 2007). The giant cane k was lower than the Miscanthus × giganteus k grown in the same experimental area (Cosentino et al., 2007).

Significant differences in KUE have been observed between double and single giant cane harvest in a typical northern Mediterranean environment. A significant effect of nitrogen fertilization on the NUE of a giant reed plantation in the long run in the northern Mediterranean gave the following results. As the giant reed grew, the NUE decreased and the amount of nitrogen increased (Monti and Zegada-Lizarazu, 2016).

The agronomic NUE in different nitrogen fertilization treatments and soil water availability in a semi-arid Mediterranean environment has changed considerably based on nitrogen fertilization treatments and the age of the crop. The effect of nitrogen fertilization on NUE was significant only in the first and second growing seasons, but not in the third growing season. This could be explained as the ability of perennial herbs to regulate and mobilize nutrients upward during the growing seasons and downward after the onset of senescence (Cosentino et al., 2016).

Several experimental field tests between giant reed and miscanthus revealed that miscanthus had a WUE higher than the giant reed. However, statistical differences between species were recorded only in the first year of growth, 4.3 g L^{-1} in miscanthus and 2.9 g L^{-1} in giant reed, while similar values were recorded in the following year (approximately 3.5 g L^{-1}).

The WUE (ratio between the dry yield above ground at the time of harvest and the cumulative evapotranspiration) was calculated with a lysimeter (Triana et al., 2014). During, 5 years field test on a semi-arid Mediterranean environment, WUE was shown to have values between 0.93–1.0 g L^{-1}, when irrigation water was supplied at 25% or 75% of maximum evaporation restoration.

This WUE increased to 5.04–7.63 g L^{-1} in the fourth and fifth year of growth, when irrigation was stopped and crops were grown only in rainy conditions a linear negative relationship was found between WUE and the water used by the crop (Mantineo et al., 2009).

Significantly higher WUE values were observed in rain (3.74-4.03 g L^{-1}) than in intermediate conditions (2.60-3.67 g L^{-1}) or well-watered (2.08-3.45 g L^{-1}). Nitrogen fertilization has led to higher WUE values; the slope of the linear regression indicated that WUE decreased by 0.18 g for every 100 mm of water use by the crop without nitrogen fertilization and by 0.19 and 0.23 g when 60 and 120 kg of N ha were supplied respectively (Cosentino et al., 2014).

Experimental research has shown close relationships between stomatal conductance and WUE, and the available content of soil water and WUE in a field test in a semi-arid Mediterranean environment. The WUE was maximum when the available water content in the soil was between 40% and 60% of the field capacity. In these soil moisture conditions, the transpiration rate decreased due to the partial closure of the stomata and the net photosynthesis remained unchanged at its highest levels with consequent improvement in the WUE. Furthermore, the water potential of the leaves has indicated the absence of water stress of the plants (Cosentino et al., 2016).

Salinity tolerance

Water stress and salinity are among the most important environmental limits that affect the growth, development and yield of plants in arid, semi-arid and Mediterranean environments (Araus et al., 2003; Munns and Tester, 2008; FAO, 2012).

The Arundo donax L., a C3 crop, in the hot season, is grown in increasing conditions of air temperature and global solar radiation, with a reduction in seasonal rainfall and an increase in the evapotranspiration potential. As regards salinity, several experimental field studies have been carried out on giant reeds in marginal environments in southern Italy (Cosentino et al., 2006).

The seedlings were transplanted into pots and irrigated with solutions of Na of 4 and 8 dS m^{-1}. Significant differences were found between the salinity levels between the different seedlings. Regular irrigation with salt water caused an increase in the electrical conductivity of the soil which reached 2.2 dS m^{-1} in the control, 6.3 dS m^{-1} in the mild and in the salinity level strict 9.1 dS m^{-1}.

This has resulted in the reduction of the main plant growth parameters (biomass yield, main stem height, specific leaf area, LAI and water content of the leaves).

However, the specific gravity of the leaves and the leaf-stem ratio showed an opposite trend. On the other hand, a slight level of salinity has led to an increase in the dry weight of roots and rhizomes. The biomass yield was reduced by 44% to severe salinity levels compared to the control theses, while the reduction was only 15.3% in the theses treated with mild salinity (Cosentino et al., 2013).

Subsequently, have been studied the effect of stress on different giant reed clones and a stress susceptibility index was used to discriminate between clones subjected to water stress and salinity stress. In this experiment, salinity levels were 16 dS m^{-1} and water stress also has been added.

It was found that the “Agrigento” clone (from southern Italy) was suitable for growth in Mediterranean areas in conditions of water stress, due to its lesser decrease in net photosynthesis, relative water content and area of green leaves. On the other side, *Arundo donax* L. “Fondachello” clone (from southern Italy) was suitable for cultivation in marginal lands where salinity stress prevailed (Sánchez et al., 2015).

Propagation

The settlement period is the most critical aspect of the cultivation of giant canes and influences the productivity and the end of life of the plants. Rhizome transplantation between late February and mid-March has been shown to be an effective method of propagation in Mediterranean areas of Europe (Copani et al., 2009). However, the main disadvantage of propagation through the rhizomes is represented by the higher costs compared to the use of cuttings and full-stemmed seedlings. The lack of effective mechanization systems for planting rhizomes is one of the reasons for the high costs. In the case of the propagation of the stem, the main problem is the low budding capacity of the buds, with consequent irregularities in the density of the plants and biomass yields lower than expected (Copani et al., 2009, 2010).

Requirements

The response to nitrogen fertilization of giant canes varies widely in different environments, growing conditions and the age of the crop. In some cases, the giant cane responds better to nitrogen fertilization only during the first 4 years after implantation; and attributed this effect to the growth of the root system (Angelini et al., 2005).

Nitrogen fertilization seems to favor the development of rhizome biomass over the years (Nassi or Di Nasso et al., 2011a, 2013). Similarly, in a semi-arid environment, fertilization is much more important at the time of planting than in subsequent years; however, it could also lead to an increase in weeds and therefore in the phenomena of interspecific competition. On the other hand, long-term studies indicated that, based on the state of the nutrients in the soil and the state of development of the roots, the response to fertilization with N gave a significant or unchanged response on the production of biomass of the crop (Cosentino et al., 2014).

Arundo donax L. is a species considered invasive in the habitats of US coastal areas or in areas near rivers and lakes. Otherwise, the European Commission has defined giant reed as one of the cheapest and most environmentally friendly crops, as approved by the new Renewable Energy Directive (RED II).

It is an invasive and resistant species capable of growing in less favorable conditions than many other plants. The distribution of the habitat of the giant reed varies from clayey and very humid soils to sandy and relatively dry soils. It has been classified as halophyte and suitable for wetlands (Lewandowski et al., 2003; Williams et al., 2009; Mann et al., 2013).

Different results have suggested the giant cane as a species that can grow equally in soils with a water content equal to the capacity of the field and in flooded soils, obtaining impressive productivity, while reducing its potential biomass production only in conditions of severe water stress (Mann et al. 2013; Cosentino et al., 2014).

Its resistance to drought is attributed to the very deep rhizomes and roots that manage to reach the aquifers. It is an excellent bioenergetic crop and numerous studies have suggested growing it on less productive and marginal soils to avoid competition with food crops or the ILUC problem. This has led to growing interest in this crop to test it under conditions of water stress or salinity. In 3 year field trial on a semi-arid Mediterranean environment, the effect of the water content of the soil available on the morphological traits and biomass yield of the giant reed was studied. In general, the density of the stem was not affected by irrigation; the height of the stem, from the elongation phase to the harvest, was significantly higher in irrigation conditions than in any irrigation or rain during the experimental period (Cosentino et al., 2014).

LAI was higher in well-watered conditions only in the maximum development phase, but it was not at the time of harvest. The yield of the dry matter from biomass was influenced by the irrigation water: the yields were 29.8%, 34.6% and 40.0% more in those well irrigated than in rainy conditions only. In the same study, an asymptotic nonlinear relationship was developed to predict giant cane biomass yields based on the use of irrigated crops in a semi-arid Mediterranean environment. The model explains well the relationship between two variables and how the yield tended to increase almost linearly up to 450 mm of water, while the increase was less than proportional to greater quantities of water. Arundo donax L. is a crop that requires a lot of water; however, its root system could allow the crop to absorb water at soil layers up to 150 cm deep in rainy conditions or up to 180 cm when irrigation water is constantly applied (Cosentino et al., 2014).

Crop protection

The Arundo donax L. is characterized by high rusticity and limited susceptibility to pathogens and insects therefore, it usually does not require chemical treatments. The leaf and the stems of the giant reed contain, among other chemical components, alkaloids and silica which improve the protection of plants against pests and predators. Furthermore, due to its large leaf mass and high growth rates, the giant cane does not undergo herbaceous competition from the second year onwards since it substantially reduces the availability of light and water to weeds (Jackson and Nunez, 1964; Perdue, 1958; Zegada-Lizarazu et al., 2013).

Harvest times

The conventional harvesting operation of Arundo occurs only once a year, generally during the winter season, when the above-ground organs are senescent. The biomass collected in this period has a good quality as a fuel thanks to the lower moisture content and the reduced concentration of harmful elements such as minerals and nutrients, which are mobilized in the rhizomes before winter (Smith and Slater, 2011). The growing interest in using giant reed for biogas production has reconsidered the collection times. In summer and autumn there are higher yields in biogas than the traditional winter harvest (Ragaglini et al., 2014). This is mainly due to the quality of the biomass obtained, whose characteristics are more suitable for anaerobic digestion than the thermochemical processes for obtaining fuels (Dragoni et al., 2015; Ragaglini et al., 2014).

The collection of Arundo donax L. is completely mechanized and can be done using different strategies. The choice of one harvesting method over another is determined by several aspects, such as the state of the crops, the moisture content of the biomass at the time of harvesting, the end use, the biomass quality parameters required, the logistics, the availability of the equipment and the type of storage. Four possible collection strategies for Arundo can be defined: shredding and loading for transport of fresh products, mulching and baling for loading and transport of fresh products, moving, pick-up, shredding and loading for transport of dry products and shredding windrowing baling loading and transport of dry products.

Currently, giant reed is grown in eastern Asia, the Mediterranean regions and the eastern and western coasts of the United States. Giant barrel production potential has been reported worldwide. A wide range of yields is reported in the literature depending on the site, climate, soil type and fertility, inputs, cultivation and harvesting practices and planting age (Lambert et al., 2014; Williams et al., 2009).

The characteristics of the giant reed for bioenergy production are its yield potential and growth rate, its tolerance to dry environments and low input cultivation. The fuel characteristics of the collected material, such as calorific value, ash, volatile substances and carbon content of the stems, can be considered satisfactory for the production of energy. The dry matter content of Arundo donax L. grown in Mediterranean climates ranges from 36% to 57%. The dry matter content is higher in single collection systems than in double collection. The ash content is higher in crops grown without fertilization and harvested in winter. (El Bassam, 1996; Christou et al., 2015; Dragoni et al., 2015). Chemical analyzes show a rather high ash content that varies from 5.3% to 8.1% depending on the clones, the year of planting and the fertilization treatment (Amaducci and Perego, 2015; Zegada-Lizarazu et al., 2010; Nassi or Di Nasso et al., 2010).

Different pretreatment processes can be performed to improve the degradability of biomass and facilitate the removal of lignin, the solubilization of the hemicellulose, the reduction of the crystallization of the cellulose and increase the surface for the enzymatic attack. Pretreatments are divided into grinding operations, heat treatment (such as the use of hot liquid water), chemical treatment (such as acid or alkaline hydrolysis) and biological treatment based on enzymatic reactions (Raspolli et al., 2011).

The pipe to emit sounds derived from Arundo donax L. can be traced back to 5000 years ago in the western world. The Egyptians seem to have used Arundo leaves to wrap mummies in the 4th century AD. Due to the multiple uses of its stems, the plant has been distributed worldwide over the millennia. It can be used to produce musical instruments, rayon, paper and pulp, particle boards, hand-woven baskets, fishing rods, fencing, shading, ornamental plants, etc. The rhizomes have been used as sudorific, diuretic, and anti-lactant, and in the treatment of dropsy (Perdue, 1958).

Giant reed is among the most productive perennial herbs in the Mediterranean environment, capable of providing constant quantities of biomass to produce energy and other plant-based bio-products. The giant reed was first studied as a raw material for bioenergy production in the late 1980s and early 1990s. In January 1997 a European “Giant Reed” (Arundo donax L.) network was established to generate information on the potential of the plant for non-food uses (energy, paper and wood pulp). In 2005 was established the network “Bioenergy chains from perennial crops in Southern Europe”[19]. The whole chain from the supply of raw materials to the production of fuel and the use of the product was developed in another EU project called BIOLYFE[20]. From

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[19] www.cres.gr/bioenergy_chains/

[20] www.biolyfe.eu

2011 to 2015, more than 25 hectares of giant cane were dedicated to specific experimental tests financed by EU projects such as OPTIMA[21]. Today other specific experimental tests financed by EU are in place like project MAGIC 2020[22] that explore the cultivation of biomass crops on marginal lands to avoid land-use competition with food production.

Giant reed is also a fuel source for producing electricity using biomass obtained from roasted giant reed (Lewis et al., 2012)[23].

It was also used to produce biogas and biomethane and for the production of ethanol. Interesting studies have shown that the giant barrel can be used to produce fuels, chemicals and other products with high added value in the context of multi-product biorefineries to ensure a sustainable transition from the oil-based economy to the bio-based one. The giant barrel has been tested as a low input sustainable raw material for the production of advanced chemicals and biofuels[24].

Miscanthus x giganteus

Miscanthus, in particular Miscanthus × giganteus, is herbaceous grass belonging to the C4 category as regards the implementation of the photosynthetic process. It is native of Eastern Asia, it is a perennial herbaceous grass that has a high yield potential of dry matter, high efficiency in the use of resources and the ability to growing in all the different

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[21] www.optimafp7.eu

[22] https://magic-h2020.eu

[23] Portland General Electric project to convert a coal-fired power plant into a facility that operates with total biomass of 300 MW of capacity (2.6 million MWh year^{-1}).

[24] www.eurobioref.org

climatic conditions. The wide geographic distribution of miscanthus in East Asia has resulted in enormous genetic diversity and consequently phenotypic variation.

Physiology

Miscanthus performs photosynthesis through pathway C4. The C4 pathway directly influences the efficiency in the use of the resources of the crop. It contributes to the high efficiency in the use of the water through a reduced evapotranspiration keeping the stomata closed longer and fixing the available CO_2 more efficiently than the path C3 (Byrt et al., 2011; Sage and Zhu, 2011).

Being a sterile clone, Miscanthus × giganteus can only be propagated vegetatively and rhizomes are mainly used. The use of seeds has advantages such as lower costs, higher propagation rates, rapid access of farmers to new genotypes and phytosanitary safety (Xue et al., 2015b).

Miscanthus × giganteus is able to perform photosynthetic activity at temperatures up to 6° C, even lower than the threshold temperature for maize (Wang et al., 2008). Despite being a C4 plant, some miscanthus genotypes are resistant to cold and can survive even harsh winters (Clifton-Brown and Lewandowski, 2000a).

Miscanthus has a high efficiency in the use of nutrients because it does not require high inputs; manages to recycle nutrients and translocate them to rhizomes. The demand for nutrients for optimal growth depends heavily on soil conditions, which is significantly lower than other C4 crops such as sorghum, sugar cane and corn (Van der Weijde et al., 2013). The efficiency of use of solar radiation varies with temperature and decreases with water stress (Hastings et al., 2009). Some miscanthus genotypes have been shown to be large water conservatories, especially in low soil water conditions; others such as the commercial miscanthus × giganteus genotype much less (Clifton-Brown and Lewandowski, 2000b).

Being a perennial C4 grass, Miscanthus offers a number of environmental benefits, one of which is the low overall use of chemicals for pest management and crop protection. However, effective weed control is very important during the first year in order to avoid negative impacts on crop success and competitiveness in subsequent years. In the first year, mechanical weeding can be carried out between the rows and, once the harvest is well rooted, on the complete field. It is important to ensure a low herbaceous competition in spring and early summer. Weeds that grow at the end of summer can be tolerated. Europe compared to East Asia, where Miscanthus is indigenous, the incidence of pests and diseases is low and only a few specific diseases of miscanthus have been reported to date. Thus, no measures are needed for the active control of pests or diseases for the production of miscanthus in Europe.

Miscanthus is a nutrient efficient crop for the efficient use of nutrients accumulating in rhizomes and for active nitrogen fixation. Fertilization recommendations vary greatly depending on soil conditions and nutrient output (Cope-Selby et al., 2017).

Various studies have shown that Miscanthus adapts very well to the typical environmental zones of central and southern Europe. Unfortunately, dry summer periods are a problem for this crop (Zegada-Lizarazu et al., 2010; Cosentino et al., 2007a). The giant reed differently to miscanthus is a drought-resistant crop therefore suitable in temperate and semi-arid environments with high temperatures and droughts typical of the summer period (Cosentino et al., 2014, 2016).

Miscanthus × giganteus is able to perform photosynthetic activity at temperatures up to 6° C, lower than the threshold temperature for maize (Wang et al., 2008). Despite being a C4 plant, some miscanthus genotypes are cold-resistant and can survive harsh winters (Clifton-Brown and Lewandowski, 2000a). The quantity of water required for each kg of biomass is lower for Miscanthus than for corn and sugar cane (Van der Weijde et al., 2013). Miscanthus achieves high efficiency in the use of nutrients even with low inputs because it recycles the nutrients accumulating in the rhizomes. This varies between the various genotypes, for example the early flowering ones complete the translocation of nutrients before the frost kills the stems (Cadoux et al., 2012) therefore it is a more efficient crop than other C4 crops such as sorghum, sugar cane and corn (Van der Weijde et al., 2013). The efficiency of use of radiation is indicated to vary with temperature and is reduced by water stress. (Hastings et al., 2009). Some miscanthus genotypes have been shown to be conservative of water, especially in drought conditions others such as the current commercial genotype Miscanthus × giganteus minus (Clifton-Brown and Lewandowski, 2000b).

Tolerance to abiotic stress

Miscanthus has proven to be productive on marginal soils including saline soils (Qian et al., 2014; Lewandowski et al., 2016). However, the commercial genotype of Miscanthus × giganteus shows limits for abiotic stresses, particularly drought (Clifton-Brown and Lewandowski, 2000b).

Therefore, the aim of the various EU projects is to identify traits and mechanisms relevant for abiotic stresses (drought, salinity, cold and frost) that affect the production of miscanthus (Lewandowski et al., 2016). Genotypes have been found that can tolerate drought compared to the commercial genotype of M. × giganteus. Salinity-tolerant genotypes that tolerate average electrical conductivity values without significant biomass losses between the M. sacchariflorus and M. sinensis types have been identified. Instead, M. × giganteus has not proved tolerant to salinity because they use a mechanism that actively prevents the accumulation of ions in the leaves and therefore minimizes damage to essential physiological processes such as photosynthesis (Lewandowski et al., 2016). In general, plants with larger rhizomes were more tolerant to salinity than plants with smaller rhizomes (Chen et al., 2017). The frost tolerance assessment revealed that there are M. sinensis and hybrid tolerant genotypes compared to the M. × giganteus genotype (Lewandowski et al., 2016).

Miscanthus has proven to be productive on poor agricultural soils, including saline soils (Qian et al., 2014; Lewandowski et al., 2016) However, the standard Miscanthus × giganteus genotype shows limitations regarding abiotic stress, in particular the drought (Clifton-Brown and Lewandowski, 2000b). Therefore, the goal of the research project is to identify traits and mechanisms relevant for abiotic stress drought, salinity, frost, which are relevant for the production of Miscanthus (Lewandowski et al., 2016).

Salinity-tolerant genotypes that tolerate electrical conductivity values of up to 2.5 without significant biomass losses between Miscanthus sacchariflorus and Miscanthus sinensis have been identified because they use a mechanism that actively prevents the accumulation of ions in the leaves and therefore minimizes damage Miscanthus × giganteus has not been shown to tolerate salinity to essential physiological processes such as photosynthesis (Lewandowski et al., 2016). In general, plants with larger rhizomes were more tolerant to salinity than plants with smaller rhizomes (Chen et al., 2017). as regards frost tolerance, there are more tolerant Miscanthus × giganteus genotypes (Lewandowski et al., 2016).

Propagation

The genetic improvement activity of Miscanthus began in Germany in the 1960s. At the end of the 1980s, species that grew spontaneously in Europe were found and breeding began to obtain bioenergy. The identification of the parents of Miscanthus × giganteus occurred thanks to Greef and Deuter in 1993. Subsequently, Miscanthus hybrids have been tested in different European environments (Clifton-Brown et al., 2001; Lewandowski et al., 2016).

All the germplasm that has been found in the areas of origin in Asia and has been used in breeding programs in Europe and the United States (Clark et al., 2015). In the UK, thousands of crossbreeds with two parents have been attempted within the same species and between species. While the flowers of Miscanthus sinensis and Miscanthus sacchariflorus have both anthers and stigma (dioecious grass), most Miscanthus genotypes are self-incompatible. Miscanthus hybrids are still being tested in several locations in Europe and the United States (Clifton-Brown et al., 2015; Lewandowski et al., 2016).

The most relevant breeding selections used frequently in the initial screening stages are made in nurseries using the vessels. The quality characteristics of the biomass are relevant when selecting genotypes for specific uses. Currently, Miscanthus × giganteus is bred in the field with vegetative propagation methods which unfortunately have high costs. Currently the genetic improvement activity is focusing on the creation of hybrids that produce seeds suitable for propagation.

The primary goal of Miscanthus genetic improvement programs in Europe has been to increase biomass yield with minimal input in different environments. New hybrids of Miscanthus × giganteus have been identified that are well suited to drought, cold or salinity (Lewandowski et al., 2016). The main criteria for evaluating the performance of bioenergy crops are the dry matter yield and the energy yield per hectare (Kiesel et al., 2017).

Production

Currently, around 123,000 ha are used for the production of miscanthus biomass globally. The largest area is located in China, mainly used for papermaking (Xue et al., 2015a), but also as a building material and for food. In Europe, there are approximately 20.000 hectares of miscanthus, mainly in United Kingdom, France and Germany. In the UK, miscanthus biomass is used to generate electricity in dedicated combustion power plants. In Germany it is used for thermal conversion in small-scale heating systems. It is also used as building materials and biocompounds. Aside from several hectares of M. sinensis used to produce straw in Denmark, only the M. × giganteus genotype is grown for commercial purposes in Europe. M. × giganteus is also produced in the United States on an estimated area of 3.200 ha.

Yields reported from field trials in Europe with the Miscanthus × giganteus standard genotype vary by location and time of harvest. In general, returns are lower on sites with water stress or other abiotic stress. They also decrease when the harvest is delayed after the maximum yield peak. However, to obtain the best biomass quality to be used as a fuel for combustion, the miscanthus is normally collected in the spring after full senescence and the transfer of nutrients into the rhizomes and has had time to dry on the field with humidity <14%.

Some EU projects investigated the productivity of new miscanthus genotypes, compared to Miscanthus × giganteus, in Europe (Lewandowski et al., 2003, 2016). Several field measurements were made in different locations to predict the productivity of miscanthus under different conditions (Hastings et al., 2009). In the OPTIMISC project, 15 miscanthus genotypes were compared with Miscanthus × giganteus (Nunn et al., 2017). The results defined the great potential productivity of miscanthus throughout Europe and also the possibility of increasing the cultivation areas both in the north and in the east.

Miscanthus is not a typical crop for the production of liquid fuel (first generation biofuels), since it does not contain extractable oils and very little sugar. Its biomass is called lignocellulosic, with a high content of cellulose and hemicellulose (cellulose + hemicellulose = holocellulose). The cellulose, hemicellulose and lignin content can be influenced by the choice of the genotype and by the time of collection of the biomass. Due to the growing number of plants for producing large-scale second-generation biofuels[25] worldwide, Miscanthus has the potential to become an important biofuel crop. (Hodgson et al., 2011; Van der Weijde et al., 2017).

The suitability of new Miscanthus genotypes for bioethanol production has been reported in the literature, indicating the great potential of this crop. In addition to the production of biofuels, biomass can be converted into chemicals for use in various applications, including bioplastic. This is currently a research and development sector, also with the aim of stabilizing the bioeconomy in the chemical industry, allowing a high value application of the Miscanthus biomass, which at the same time can contribute to guaranteeing the growing biomass demand for a growing bioeconomy (Kärcher et al., 2015, 2016; Van der Weijde et al., 2017).

According to the genetic variety, it can present a different production of biomass and a different potential use. The biomass produced by Miscanthus can be used for energy purposes (combustion, biogas and liquid fuels), to produce materials and chemicals (building materials and bedding for animals) and for food use. It has all the characteristics of Perennial crops cultivated in marginal lands. Miscanthus can improve all those aspects related to biodiversity, to improving the soil structure and the biogeochemical cycles that take place in the environment. Obviously, it must be considered that, as for all Perennial crops also for Miscanthus, the production costs and the reduction of greenhouse gases such

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[25] Second-generation biofuel refineries can be classified according to the conversion process used: (1) thermochemistry (gasification and pyrolysis) and (2) biochemistry.

as CO_2 decrease significantly.

Being a perennial grass with a high yield potential, Miscanthus × giganteus is a promising crop for the supply of large quantities of low-cost biomass for use in the anaerobic digestion process. In Europe, Miscanthus × giganteus is among the most productive genotypes and is also the only commercial variety available. It is used to obtain biogas harvested still green in October. Milling is a pretreatment that can significantly influence the specific yield of methane and the speed of production of the methane itself. For this reason and to avoid interferences in the process, an adequate pretreatment of the biomass of Miscanthus × giganteus is recommended. Various pretreatment technologies have been described in the literature, such as extrusion, grinding, ultrasound and treatments with white and brown mushrooms (Frydendal-Nielsen et al., 2016; Patinvoh et al., 2017).

Saccharum spontaneum L.

Saccharum genus, Saccharum spontaneum L. spp. aegyptiacum (Willd.) Hack. (common name African fodder cane) is a C4 perennial, herbaceous, rhizomatous grass, native from North Africa and widespread in South Mediterranean regions. Saccharum genus has 40 species mostly native to South-Easthern Asia (Clayton and Renvoize, 1986), high polymorphism, robust and resistant to physiopaties (Pignatti, 1982).

African fodder cane is a lignocellulosic, perennial, rhizomatous, no-food crop that grows spontaneously in the semi-arid Mediterranean area.

This species has all those agronomically desirable characteristics in a biomass crop. It is a C4 grass, it has a high biomass yield, a high assimilation rate even during periods of water stress, the ability to use water efficiently and a satisfactory biomass quality (Cosentino et al., 2015b).

Hovewer, the research is needed to look for plants that still conserve those genetic traits associated with growth under drought stress and severe water stress. This research would evaluate the potential yield of perennial C4 grasses used as bioenergy crops, while biomass quality growing in the Mediterranean area. The Mediterranean environment have some characteristics that might foster growth perennial C4 grasses like active temperature >10° C, incoming solar radiation, length and growing season and other limiting factors that could reduce biomass production in perennial C4 grasses like low rainfall and drought in spring-summer (Cosentino et al., 2007, 2014b).

Research looking for plants that could growing on soils affected by water deficit or other constraints like salinity. Saccharum spontaneum L. spp. Aegyptiacum (WIlld.) has a range of aqgronomically desiderable traits of biomass crop. Is a C4 plant, has a high biomass yield, an active assimilation rates during drought-stress periods and a high water use efficiency.

In the Mediterranean area, likely that in arid and semi-arid regions, where evapotranspiration dominates is reduced water availability, and duration of drought may increase (Cosentino et al., 2012). It is very important to investigate for drought tolerant lines as water resources are becoming limiting and for marginal soil also with high salt content. It has been shown that C4 perennial grasses are able to use abiotic resources, mainly water, more efficiently than C3 species (Long, 1999; Triana et al., 2014).

Soil water availability have determined a significant increase in stem height in all growing seasons. It was shorter in rainfed that intermediate and fully irrigated treatments. The higher stem was registered in the first growing season and there is no difference in basal stem diameter. Stem density did not show any difference between treatments within growing season. Dry matter yield was significantly affected by soil water content and the crop WUE as result of rainfall, irrigation and soil water content, showed that was higher in the first growing season than in the second and in the third ones. Moisture content was highest in the fully irrigated treatments and lowest in rainfed condition, but ash content increased when irrigation was reduced. Fiber content (hemicellulose, cellulose and lignin) did not show any difference amongst irrigation treatments (Cosentino et al., 2015).

In arid and semi-arid Mediterranean regions, where evapotranspiration dominates, reduced water availability and duration of drought. For Mediterranean area, it is very important investigated for drought tolerant crops because water resources are becoming limiting (Cosentino et al, 2012). Clearly, C4 perennial grasses are able to use abiotic resources, mainly water, more efficiently than C3 species (Triana et al., 2014). In a recent study about Saccharum spontaneum L. spp. Aegyptiacum resulted that the Δ^{13} C (-13.19%±2.3%) of under well-watered conditions is similar to other annual and perennial C4 plants like corn, sugarcane and dryland grasses (Cosentino et al., (2015). Terrestrial C3 plants have a Δ^{13} C that average -26.7±2.3% on global scale (Cerling et al., 1997).

African fodder cane possesses a range of agronomic traits typical of biomass crops: C4 plants, rapid grow rate, high biomass yield, few no natural enemies, active assimilation rates during drought-stress, able to use water efficiently. The higher yield of Saccharum spontaneum spp. Aegyptiacum that Arundo donax and Miscanthus × giganteus in semi-arid Mediterranean area might be related to the longer vegetative phase of the former.

Stomata regulation and reduced transpiration have been reported as mechanism for drought avoidance for several crops (Clifton-brown and Lewandowski, 2000b). Similarly, to Arundo donax also Saccharum spontaneum has a leaf rolling increasing avoidance of dehydration by reducing incident radiation on leaves, leaf temperatures and water loss (Cosentino et al., 2014b).

Recently, in a comparative study with the use of a lysimeter system the C4 Miscanthus × giganteus and the C3 Arundo donax have shown higher crop WUE range respectively 3.7-4.3 gL^{-1} and 2.9-3.2 gL^{-1}. The crop WUE of Saccharum spontaneum spp. Aegyptiacum showed a range of 3,24gL^{-1} under high water imput treatments and 5,98 gL^{-1} with low water input treatments. These values were higher than Arundo donax and Miscanthus × giganteus in semi-arid Mediterranean field conditions (Cosentino et al., 2015).

The characteristics that influence the suitability of this herbaceous perennial crop to be used for thermochemical and biochemical conversion were determined.

Increasing the amount of irrigation water decreased ash content, bulk density, CHN composition and LHV and increased moisture content and ash melting point. The IDT and MCRT and fiber content were not influenced by increasing irrigation water content. For thermochemical pathaways an high ash and moisture content have negative influence on the bioconversion efficiency (Jenkins et al., 1998).

For biochemical conversion fiber content was comparable to other monocot species as Arundo donax and Miscanthus × giganteus and other like corn stover, wheat straw, rice straw and switchgrass (Scordial et al., 2014). Other studies reported that Saccharum spontaneum spp. aegyptiacum has a structural polysaccharides composition ideal for second generation bioethanol production (Scordia et al., 2010; Cosentino et al., 2015).

Bioenergy and Bioproducts

The EU Renewable Energy Directive (RED) has provided the use of food crops and / or feed crops for the production of first generations biofuels. Currently, has been implemented so completely different from the previous ones to block the use of food or feed crops to produce fuels for the consequences that derive from them such as changing soil use and increasing of greenhouse gas emissions.

Consequently, both in Europe than in the other Countries all over the World, it has been possible to observe a growing interest in those Perennial crops, which grow spontaneously in marginal and / or degraded lands, as a source of Biomass for energy production.

The spontaneous Perennial crops that have received the most interest from the international science were lignocellulosic crops because they have low cost of raw materials and are able to grow in different environments and in particular on marginal lands.

They are the raw materials most used to produce a series of products with high added value such as: biopharmaceuticals, nutritional supplements, biopolymers, biomaterials, construction products, soundproofing materials, mulching, biodegradable products for gardening, animal bedding, advanced biofuels, heat, energy, organic soil fertilizers and green chemicals. All these products with high added value are the basis of the modern economy called “biobased”.

However, research is still directed towards on the best agronomic techniques for growing them, on post-harvest logistics and on bioconversion. Furthermore, new elite varieties are being studied to expand in the European and World market to achieve the highest yield potential and a better quality of the Biomass obtained from these Perennial crops, while at the same time seeking to maximize the conversion efficiency.

Renewable energy from biomass derives from any available and renewable organic material used as it is or transformed into solid, liquid or gaseous energy carriers through the thermochemical or biochemical conversion of structural carbohydrates, lignin, proteins, fatty acids and other chemical components into soluble compounds (IEA, 2002).

The Perennial or herbaceous low starch energy crops includes species such as miscanthus (Miscanthus spp.) and giant reed (A. donax L.). Non-food lignocellulosic perennial crops can replace existing crops also used to produce food and feed in the production of biofuels and bioenergy. this would reduce competition between food and fuel, competition for the intended use of soils and therefore favor the restoration of severely degraded and / or heavily contaminated soils (Zegada-Lizarazu et al., 2010).

Perennial crops belong to the Poaceae or Gramineae family, the largest form of vascular and herbaceous monocotyledon plants, which include cereals, natural, semi-permanent and permanent grasslands, meadows and bamboo. In general, herbaceous plants are classified into annual species (cereals) and perennial species (fodder etc.). Perennial crops are currently the most common plants. Perennial crops are a valuable source of food, feed and energy for all types of wildlife, pets and humans (Piperno and Hans-Dieter, 2005).

In general, Perennial crops are drought-resistant crops and lately their interest from the global community and agriculture has grown over time because it has been shown that they can be used as an ideal raw material to obtain Bioenergy and Biobased Products.

Compared to traditional crops for the production of food or feed Perennial crops, in particular spontaneous ones, do not require energy inputs (fertilizers, pesticides, etc.), can be grown on marginal lands and also improve the structure, contributing to keeping the soil structure stable preventing tipical phenomena such as erosion and runoff. They also improve the soil quality they increased its fertility (organic substance and nutrients readily available for crops in the circulating soil solution) and biodiversity. Perennial crops, especially spontaneous ones, do not enter in competition for the use of agricultural soil for the production of food and feed crops, because they can be grown on marginal or degraded lands. Perennial crops have been used for centuries as fodder crops for animal feed, also contributing to energy supply on farms. first in the United States and then in Europe since the mid 1980s (Lewandowski et al., 2003) there has been growing interest in the use of perennial crops to produce biomass. Several European projects on the study of perennial crops to produce energy have been implemented (Miscanthus, Giant reed, Switchgrass) projects with the name of 4FCROPS and EUROBIOREF (Alexopoulou et al., 2015).

In particular, the European Commission has funded three research projects: “KBBE.2011.3.1-02” on perennial crops, “SICA” for biomass production, “OPTIMA”, to experiment the cultivation of Perennial crops in marginal lands and the main results are found in the proceedings of the Conference “Perennial organic crops for a limited world of resources”[26]. Perennial crops for biomass production are still largely non-domesticated plants. Most grow spontaneously are therefore wild plants and are collected to be able to test them with scientific experiments in test fields and to be able to observe their physiological behavior in the various breeding phases up to the harvest. Therefore, the appropriate varieties, their agronomic practices or post-harvest logistics have not yet been optimized to achieve potential yield in a given environmental condition. However, wild germplasm retains all those specific characteristics of resistance and phenotypic plasticity therefore able to adapt to the most varied biophysical constraints typical of marginal lands. This germplasm is used for reproduction programs and to test its efficacy also in contexts other than that of origin (Zegada-Lizarazu et al., 2010).

The main objective pursued with the various European

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[26] www.biomass2015.eu

projects on the use of perennial crops for the production of biomass in particular in marginal soils is to be able to achieve maximum production (biomass yield, energy content) by minimizing inputs (tillage, fertilization, irrigation, weeding and pest control, harvesting, transport, storage, pretreatment and bioconversion to energy consumption).

From the various field experiments it has been observed that cultivation techniques and post-harvest must be improved. The ideal would be to be able to provide genetically identified and mapped “ideotypes” of bioenergy crops suitable for different environmental conditions.

The European continent extends over a vast geographical area, which borders Norway to the north with Greece to the south, Iceland to the west and the Ural mountain range to the east. This means a great variety of climatic environments in fact according to the European Biodiversity Observation Network (Wageningen University & Research) the European territory has been classified into 13 different environmental zones (Metzger et al., 2005; Jongman et al., 2006).

The main climatic parameters were observed (minimum and maximum annual average air temperatures, quantity and distribution of rainfall, number of months with temperatures that prevent plant growth, duration of the growing season, cumulative growth degree days below above a basic threshold temperature of 10° C) to be able to classify the entire European territory in 13 different environmental zones and to be able to distinguish also those zones suitable for the cultivation of biomass crops for bioenergy production. From these 13 different environmental zones, 8 suitable environmental zones have been designated for the cultivation of biomass crops right for bioenergy (Cosentino et al., 2012).

The northern and continental climatic zones delay the emergence of plants (perennial crops) due to the spring frost and cause an interruption in the growth in autumn and this negatively affects the biomass yield.

The temperate oceanic climatic zones, where the summers are cool and with a low luminous intensity, the plants have only the long spring-summer days to grow and therefore be able to obtain a high biomass yield.

The northern Mediterranean environment instead shows very favorable climatic conditions in the spring-summer period, presenting a high luminous intensity and consequently promoting the growth and high yield of the plants.

The southern Mediterranean environment has very favorable climatic conditions from spring to autumn. High summer temperatures can shorten the growing season, although plants can benefit from it to continue growing (Scordia et al., 2014).

However, severe water stress occurs for plants that usually lasts from 2 to 6 months (Neùeman and Goubitz, 2000) and short periods in the absence of rainfall from autumn to spring limit the growth of plants and consequently the production of biomass (Cosentino et al., 2007a; Gulías et al., 2009).

According to the general lines of plant physiology, the rate of growth and development of plants depends on the growth temperature of plant, each species has its own specific temperature range represented by a minimum, a maximum and an optimal range (Hatfield and Prueger, 2015).

The air temperature influences all the physiological processes of plant growth such as photosynthesis, respiration, perspiration, protein synthesis and translocation, and consequently also the yield in biomass (Cosentino et al., 2016).

At high temperatures, the enzymatic activity and most of the chemical reactions increase and also the translocation of the photosynthesis products is faster, therefore the plants first ripen the fruits (Beven et al., 1979). At excessively high temperatures, however, a denaturation process of enzymes and proteins occurs, causing a phenomenon known as heat stress (Mader, 1993).

On the other side, excessively low temperatures can irreversibly damage the cell walls of plants (Devlin, 1975). Air temperatures above the base threshold (Tb) affect the number of days of the growing season and cumulative days of growth (° Cd).

This data determines the beginning and end of the growing season and for this reason it is used to determine the physical state of plant development in the predictive models of plants (Hastings et al., 2009).

The days to grow for a plant (growing season) represents the period of time available to grow in a specific environment. Unfortunately, there may be other factors that will limit its growth: environmental (light intensity, light quality, day duration, water stress, thermal stress, vapor pressure deficit, relative humidity, etc.), physiological (CO_2 absorption, stoma conductance, perspiration, stomatic limitation of CO_2 absorption, electron transport speed, etc.) and phenological (beginning of flowering, senescence, maturation, etc.).

Water directly or indirectly participates in all metabolic processes in living organisms. Excess water in the soil can damage plants due to lack of oxygen, causing oxygen stress, therefore a phenomenon called hypoxia or anoxia.

The shortage of water during plant growth causes water stress, affecting all the physiological activities of plants, such as photosynthesis, cell growth, leaf expansion rate and other morphological changes (Sánchez et al., 2015; Cosentino et al., 2016).

Plants react to water stress closing the stomata to limit the loss of water through transpiration (Lawlor and Cornic, 2002; Flexas et al., 2007; Cosentino et al., 2016).

The quantity and distribution of rainfall during plant growth seasons are perhaps the most important environmental factors because they affect the growth, development and yield of plants (Araus et al., 2003; Sánchez et al., 2015).

Perennial grasses are herbaceous, lignocellulosic plants ideal to obtain energy or other “alternative” uses for the modern economy called “bio-based” that Europe wants to implement.

Their chemical composition consists mainly of structural polysaccharides (cellulose and hemicellulose) from lignin and small fractions of non-structural components (extracts, proteins, lipids, pectin and ash).

Lignocellulosic crops are, by their nature, resistant to pests and diseases. This feature, however, limits the hydrolysis of structural carbohydrates for biochemical conversions, to obtain second generation bioethanol and for anaerobic digestion.

On the other hand, they are much more suitable for thermochemical conversions. For both bioenergy and biomaterials, Perennial grasses offer environmental advantages by contributing to the reduction of CO_2 and energy consumption.

However, their production costs are affected by the yields. The sustainability of the production of Perennial grass must be considered, taking into account environmental, economic and socio-economic aspects.

Perennial grasses have different characteristics based on the photosynthetic process which can be of the C3 type (A. donax, P. arundinacea, Phyllostachys spp.) Or the C4 type (Miscanthus spp., P. virgatum), according to the water needs, or to thermal needs (Lewandowski et al., 2003).

The average minimum and maximum annual temperatures of the air increase while the number of months with temperatures below 0° C and the amount of precipitation between the various environmental zones of the European territory decrease from North to South.

The distribution of rainfall during the season plant growth is variable and can occur very regularly from areas of central and northern Europe or irregularly in southern areas.

The response of plants to different environmental conditions of growth determines the selection of the most suitable crops for the different environmental zones.

Furthermore, fundamental factors such as the type of soil, the slope or other characteristics of the soil could limit or favor the choice of the most suitable crop (Cosentino et al., 2012).

Obviously, there is no perennial crop suitable for all climatic conditions (Mitchell et al., 2016), but despite the environmental conditions in which perennial crops are grown, the ideotype (ideal crop type) of biomass culture should have the following characteristics (Cosentino et al., 2007b):

- High biomass yield, as close as possible to the potential yield in a given environmental zone;
- Stable yield of biomass in changing climatic conditions;
- High efficiency in the use of resources (solar radiation, nutrients and water);
- Resistance to parasites;
- High competitiveness with infesting herbs during planting;
- Resistance to abiotic stresses (dryness, high or low temperatures, excess humidity or deficit in the soil);
- Growth capacity even in adverse biophysical conditions (unfavorable soil texture, reduced depth, saline solution, contaminated soils, steep slopes);
- Finding low cost (per seed) and low external input propagation material (soil tillage, fertilization, irrigation, weed and pest control, harvesting);
- Suitable for the use of existing agricultural equipment;
- Stable biomass quality for specific end uses.

Environmental sustainability

Perennial grasses, like all plants on Earth, play an important role in reducing CO_2 emissions because they absorb it from the atmosphere through the biochemical process of photosynthesis and transform it into organic substance. In the case of Perennial crops their importance lies in the fact that they are able to produce through photosynthesis large quantities of biomass very useful as an energy source and therefore sustainable for the so-called “bio-economy”.

In addition, Perennial crops improve soil structure (reduce erosion and runoff), soil quality (increase soil fertility in terms of accumulation of organic matter and therefore of nutrients present in the circulating soil solution and readily available for crops) and biodiversity (Lewandowski et al., 2003).

The socio-economic benefits lie at the basis of the “bio-economy” because the Perennial crops are able with their biomass production to provide an alternative energy source to the traditional energy source from fossil reserves energy that now running out from Earth. The Perennial crops allowed the repopulation of abandoned rural areas, created new forms of employment and promoted the use of marginal land unusable for food and feed crops. This means environmental sustainability of Perennial crops that are in most cases spontaneous crops (Soldatos et al., 2010).

The development of the “bio-economy” with the production of biomass crops such as perennial crops on marginal land in the EU to produce bioenergy is the main objective to be achieved the poorness of fossil in the European lands and unfortunately EU needs to purchase energy from other non-EU countries (Scarlat et al., 2015).

Sustainable agriculture defined “producing more outputs in the same hectares of land to reducing negative environmental impacts and to improving the natural environment and services” is the basis of sustainable development because the whole World economy starts from the primary sector which is agriculture (Pretty et al., 2011).

Unfortunately, the EU needs to increase biomass production despite the fact that the lands available for the production of Perennial crops is very small and this will cause unsustainable use of water, fertilizers and pesticides (Alexopoulou et al., 2015).

For this reason, the research is aimed to the use of spontaneous and very resistant Perennial crops, resistant to biotic and abiotic adversities, suitable for growing in marginal lands without further inputs such as irrigation or the use of fertilizers and pesticides.

Life cycle assessment of Miscanthus × giganteus, Arundo donax L. and Saccharum spontaneum spp. aegyptiacum in the Mediterranean marginal lands has shown that the cultivation of perennial crops and their use for heat and energy generation can achieve substantial GHG emissions and significant energy savings. Perennial crops have fundamental characteristics that allow, with their production of biomass, a high yield of heat and energy, also they improve the structure of the soils, reduce the risks of erosion and runoff and also avoid the problem of changing the use soils (ILUC) (Schmidt et al., 2015; Rettenmaier et al., 2010).

The key role of Perennial crops in terms of soil erosion mitigation has been well documented (Wuest et al., 2006; Cosentino et al., 2008; Feng et al., 2011).

The Miscanthus × giganteus but also the giant cane contains very well the soil losses due to erosion compared to, for example, durum wheat or annual crops sown in autumn or uncultivated land in Mediterranean areas with soils with a slope of 26%-28% (Cosentino et al., 2015a).

Perennial crops store CO_2 in the soil because they release the plant residues of their biomass into the soil. All these environmental benefits could also be an income opportunity for farmers, because the EU rewards producers of biomass crops for energy production with "environmental credits" (Nocentini et al., 2015).

It is widely recognized that monoculture has negative effects compared to a natural system (Mattsson et al., 2000) and more forests decrease into monoculture more serious will the impact on biodiversity (Paine et al., 1996) because this influences the structure of ecological units and the native populations (Biewinga and van der Bijl, 1996; Rodrigues et al., 2003; Slootweg and Kolhoff, 2003; Smeets et al., 2009).

From the experiments carried out emerged that the impact on biodiversity from comparison between spontaneous perennial crop systems with a natural forest and annual cultivation systems in European territory, the natural forest has no impact on biodiversity (Fernando et al., 2015).

Perennial crops determine only a low impact on soil biodiversity compared to annual crops (Borjesson, 1999; Boehmel et al., 2008; Prochnow et al., 2009; Werling et al., 2014), promote the biodiversity and turn on of fauna and soil microorganisms (Borjesson, 1999), provide shelter for invertebrates, birds and small mammals (Smeets et al., 2009; Bellamy et al., 2009; Semere and Slater, 2007a, b).

Experimental part

Introduction

The present experiment is part of the MAGIC 2020 project, a research project funded by the European Commission in the frame of the H2020 Program. Nowadays, there is a need to develop new energy sources that will be able to assume the increasing global energy consumption, which is set to reach 32% in Europe by 2030[27].

The European Union is interested to developed sustainable agricultural systems to produced non-food energy crops using marginal lands and low input cultivation techniques (Cosentino et al., 2012). Moreover, there is a tendency for these new energy sources to be respectful of the environment as a way to slow the progression of negative effects of climate change (drought, sea level rise, global warming).

At present, plants that are able to grow in degraded areas, including also water stress, heat, cold and soil salinized, are being extensively investigated as bioenergy perennial crops. Indeed, the cultivation of energy crops on arable land has raised a number of concerns regarding land use change, the agricultural soils for food and livestock crops are not used, because these crops should be grown on the so called "marginal lands" (Mantineo et al., 2009) to ensure biomass production without encroaching on agricultural lands (Cosentino et al., 2014).

Soil salinization is a severe environmental stress, that limits the productivity of agricultural crops. Although the

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[27] Renewable energy directive 2018/2001/EU

amount of salt affected land (about 9 million hectare) is imprecisely known, its extent is sufficient to pose a threat to agriculture since most plants, and certainly most crop plants, will not grow in high salt concentrations. Only halophytes (by definition) grow in concentrations of sodium chloride higher than about 400 mM. Consequently, salinity is a threat to food supply (Flowers et al., 1997; Munns et al., 2002). Salinization could commonly occur to agricultural practices associated with irrigation, and nearly 50% of irrigated soils in the world, approximately 230 Mha are someway salt affected. Not only irrigation but also sea water incursions into rivers or aquifers present in coastal areas could be a source of salinization (Rengasamy et al., 2003).

Soil salinization occurs frequently in arid and semi-arid regions, however, soil salinity also widespread in humid regions such as South and Southeast Asia and affects large areas of cultivated land in more than 100 countries (Rengasamy, 2006).

Increased soil salinity negatively affects the growth of many crop plants, and the continued salinization of arable land provides an increasing threat to global crop production, especially in irrigated systems (Munns et al., 2008).

The Na^+ toxicity of many crop plants is correlated with over accumulation of Na^+ in the shoot (Munns, 2002; Møller and Tester, 2007). The Na^+ is taken up from the soil by the plant root system and transported to the shoot in the transpiration stream. Shoot Na^+ accumulation is the net result of distinct Na^+ transport processes occurring in different organs and cell types, and each of these processes contributes to the salinity tolerance of a plant.

According to Munns (Munns et al., 2002) physiological plant responses to water stress and salt stress have much in common; however, the mechanisms are extremely complex and vary with plant species as well as with the degree and time of exposure to stress. Photosynthesis, together with cell growth, has been reported among the primary processes affected by salinity or water stress (Chaves et al., 2009). Decreases in the photosynthetic rate under both stresses may be directly associated with a decrease in CO_2 availability related to stomatal closure (Flexas et al., 2007), or be due to alterations of photosynthetic metabolism (Lawlor et al., 2002). Therefore, photosynthetic performance involves a highly complex mechanism, where limitations are taking place at different sites of the cells and leaves are interacting on different time scales. At the same time, greater control of transpiration water loss is achieved to reducing the leaf expansion rate, preventing dehydration (Liu et al., 2002) and acting as the first step in the process of stress acclimatization (Chaves et al., 2009). Changes in plant morphological components have also been reported; for example, a decrease in the leaf area ratio (LAR) and specific leaf area (SLA) (Erice et al., 2010).

Stress tolerance of a plant species is usually determined by the plant's genes and also by morphological, phenological, physiological and biochemical traits. Therefore, measurements of different physiological processes during the plant's response to stress provide important information about the mechanism of the plant that are intended to remove or to reduce the harmful effects of stress in the plant tissues. Increasing the salinity tolerance of crop plants will provide an important contribution to the maintenance of crop yields (Grzesiak et al., 2013). On the other hand, another problem is the intensity and duration of stress that occurs across the globe especially in the Mediterranean region (IPCC, 2013; Cosentino et al., 2012).

The present study will focus on soil salinity stress, evaluating the potential of three selected perennial lignocellulosic crops to increasing salinity levels: as C_3 the *Arundo donax* L., as C_4 the hybrid *Miscanthus* x *giganteus* and the *Saccharum spontaneum* L. spp. *aegyptiacum* (Willd.). The crops tolerance for salinity stress will be tested for two years, applying a completely randomized design in three replications. A possible strategy, the fertilization with a NPK fertilizer, has been used and the interaction between species and salinity stress with fertilization is ascertained.

Material and methods

The present work consists of two independent experiments, named Experiment 1 and Experiment 2, both involving perennial bioenergy grasses under soil salinity and fertilization levels. In Experiment 1 rhizomes were used to propagate perennial grasses, which were grown in a clay soil under open air. In Experiment 2 stem node cutting were used to propagate perennial grasses, the soil was of volcanic origin and crops were grown under semi-open air in a glasshouse which was open in the four sides.

Plant Material

The study crop species used both in Experiments 1 and Experiment 2 are lignocellulosic, perennial, herbaceous crops. Three different species were compared: *Arundo donax* L. local clone Fondachello (Cosentino et al., 2006), *Miscanthus* x *giganteus* Greef et Deu. (Piccoplant Oldenburg, Germany) and *Saccharum spontaneum* L. spp. *aegyptiacum* (Willd.). All species were collected from the experimental fields of the University of Catania, Italy (10 m a.s.l., 37°24'N, 15°03'E).

The following factors were studied in a split-plot experimental design with three replicates both for Experiments 1 and Experiment 2: species in three levels, salinity in three levels and fertilization in three levels (*n=81*).

The substrate selected in Experiment 1 is a clay soil ascribable to the order of Entisols (U.S. Soil Taxonomy) with an alkaline reaction. These soils form on soft rocks and are mainly found on the clay formations of the Sicilian hill and in the belt that runs along the Jonian Sea (Piana of Catania).

The substrate selected in Experiment 2 is a characteristic soil of volcanic areas where they evolve on lava and pyroclastic materials. They are found mainly on Etna and also in the smaller islands that surround Sicily. They often have excellent agronomic characteristics: low density (they are soft), high exchange capacity, high water retention capacity and subacid reaction. Their agronomic potential is good unless it is limited by altitude or excessive stony. In Soil Taxonomy they are placed in the order of Inceptisols (Yong and Warkentin, 1975).

The salinity treatment, in three NaCl levels, was the same in both the Experiment 1 and 2: tap water for S_0, 9 dS m^{-1} for S_1 and 18 dS m^{-1} for S_2. At each irrigation NaCl has been added to 13,5 liter of tap water for a total amount of 121.5 g of NaCl for S_1 and 243 g of NaCl for S_2, adjusted after checking the water EC at each irrigation. Irrigation was carried out twice a week only with tap water from the moment of transplanting of rhizomes (Experiment 1) or node cuttings

(Experiment 2) into the pots up to the stem elongation phase. When the plants reached 4-5 leaves, the specific amount of NaCl was added to tap water only for S_1 and S_2.

The fertilization treatment, in three level of NPK 18-18-18, was the same for both Experiments 1 and 2: 0 kg ha^{-1} for F_0, 60 kg ha^{-1} for F_1 and 120 kg ha^{-1} for F_2. The amount of fertilizer indicated in the experimental plan was distributed before transplanting the rhizomes (Experiment 1) or node cuttings (Experiment 2) in each pot for 60 kg N ha^{-1} and 60 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1} as 18N-18P-18K fertilizer for F_1 and 120 kg ha^{-1} for F_2, respectively. After the stem elongation stage, the calculated amount of 18N-18P-18K fertilizer only for F_2 remaining quantity was applied. Fertilizer amount is shown in the **Table 1**.

Table 1. The fertilizer added as basic dressing.

Data	**Unit**
Qc[28] = 60 kg ha^{-1} / 0.18	333.3 kg ha^{-1}
Pot area (25 cm diameter)	0.094 m^2
Fertilizer 18N – 18P – 18K	0.0314 kg m^2
Qc Pot	31.3 g m^2

Weeds were controlled manually throughout the experimental period.

The pots have been arranged in an open space at the Department Di3A of the University of Catania (Italy) for the Experiment 1 and in a greenhouse at the Experimental Farm of the University of Catania (Italy) for the Experiment 2.

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[28] Qc = Amount of fertilizer.

Experiment 1

The Experiment 1 was carried out in the early spring (May 2019) at the “Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A)”, University of Catania, transplanting rhizomes in previously prepared pots in open air conditions (**Figure 1**).

Figure 1. Experimental layout in the salinity stress trial in the Exp. 1.

Fresh rhizomes have been weighed and selected with 2-3 main buds (**Figure 2**) than directly transplanted at 1 rhizome per pot, which size was 25 cm for inner diameter containing approximately 10 kg of clay soil collected from the Experimental farm (University of Catania) which was previously ploughed in winter, and then disk harrowed in early spring in 2019.

Figure 2. Rhizomes selected in the Laboratory.

In order to reduce the heterogeneity at the beginning, before transplanting in May 2019, rhizomes were selected with a similar weight where it could be possible. The initial fresh weight of the rhizomes changed between species with values ranging from 115.1 g for the *Arundo donax* to 24.2 g for the *Miscanthus* x *giganteus* and 28.7 g for the *Saccharum spontaneum* spp. *aegyptiacum*.

Experiment 2

The Experiment 2 began with plant production from stem node cuttings in Autumn (October 2019) in the nursery at the Experimental farm of the University of Catania. When plant hardening was observed, 27 plant per species were directly transplanted in pots of 25 cm in diameter and 10 liters of soil capacity. The substrate was a volcanic soil typical of the marginal lands present in the Mediterranean area and belonging to the Inceptisols category (U.S. Soil Taxonomy).

Plants were transplanted in March 2020 using the same three species, the same salinity levels and the same fertilizer levels as in the Experiment 1.

In the **Figure 3** is shown the potted plants from nodal stem cutting in the nursery of the Experimental farm at the University of Catania in October 2019.

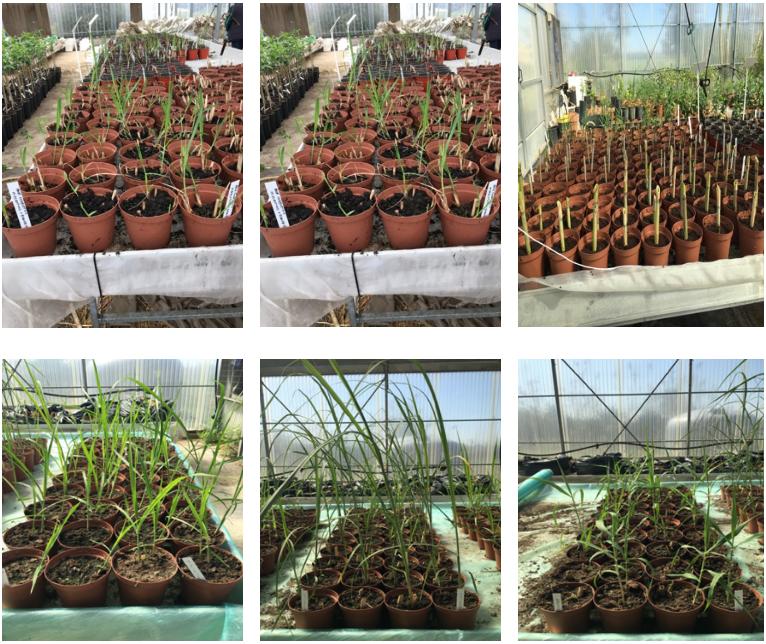

Figure 3. The lignocellulosic species selected and propagated from nodal stem cuttings in the nursery.

Stem cutting method has proved to be as successful as rhizome propagation (Copani et al., 2013; Scordia et al., 2015). Therefore, the goal to use node cuttings was to reduce the considerable variability observed with rhizomes in the previous Experiment 1.

The figure 4 shows the pot allocation in the greenhouse.

Figure 4. Experimental layout in the salinity stress trial in the greenhouse.

Measurements

Measurement of electrical conductivity (EC) on soil in the pots have been performed with GS3 Sensor ProCheck (Decagon Devices, Inc.). Soil EC was measured at the beginning of the treatment (T0) and then every week from the start of treatment with tap water plus NaCl on the S_1 and S_2 treatment, and up to one month before the biomass harvest time.

The instrument (XS COND 6+ EUTEC Instruments) was first calibrated, then the salinity of the water at the source was measured and finally the dose of NaCl to be added was weighed (g) to obtain irrigation water with the desired EC level for the respective S_1 and S_2 levels of the experiment.

Morphological measurements like basal stems diameter (mm), number of green and dry leaves, number of stems, main stem height from the base up to last node (cm), number of stems, and non-destructive leaf area index (LAI) were measured fortnight. Non-destructive LAI was calculated according to the equation developed for maize:

$$LAI = (L \cdot W) \cdot A$$

Where L, W and A are leaf length, leaf maximum width and a constant (A = 0.73) respectively (McKee, 1944).

Physiological measurement like net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}), and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) using a portable photosynthesis system (LICOR 6400 system, LI-COR Bioscience) was set on the third last fully expanded leaf using at the moment of maximum intensity of solar radiation, from 12:00 to 14:00, at a flow rate of 500 mL min^{-1} and ambient CO_2 concentration. Measurements were carried out from the stem elongation phase and up to biomass harvest. The instantaneous water use efficiency (iWUE, μmol CO_2 H_2O^{-1} m^{-2} s^{-1}) was thus calculated as net photosynthesis to the transpiration rate at each measurement time.

In the Experiment 2 only, the maximum efficiency of photosystem II (Fv/Fm) was recorded using a Hansatech FMS-2 (saturating pulse of 10,000 μmol m^{-2} s^{-1}) and dark adaptation clips (Hansatech, King's Lynn, UK) after 30 min of dark adaptation and exposure to actinic light of 2000 μmol CO_2 m^{-2} s^{-2} for a minimum of 10 min after the first saturating pulse.

Biomass harvest took place after one month of salinity irrigation treatment, collecting both the aboveground and the belowground biomass. The measurement parameters were: fresh matter yield (leaves and stems), basal stems diameter (mm), total number of green and dry leaves, number of stems, main stem height from the base of the cut up to last node (cm) and leaf area index (LAI).

After drying sub-samples in a stove at the temperature up to 65° C constant weight, the weight of dry leaves and dry culms was detected (g). For the belowground biomass the following parameters were detected: weight of rhizomes (g), roots weight (g) and number of buds.

Biomass quality

Oven-dried samples were ground through a 1-mm screen in an IKA mill (IKA-WERFE, Gmbh & Co., KG, Staufenim Breisgau, Germany). Cellulose, hemicellulose, acid detergent lignin (ADL), proteins, lipids and ash were determined by a near-infrared spectrometer (NIR, SpectraStar™ 2500XL-R, Unity Scientific) provided with a tungsten halogen lamp as light source and a high performance ultra-cooled InGaAs extended range detector. Samples were placed in small powder cups and scanned in duplicate in diffuse reflection measurement mode, wavelength range of 680-2500 nm and accuracy <0,1 nm. A previous calibration developed by the Ucal complete chemometric calibration software (InfoStar 3.11.0 version) was adopted. The calibration consisted of a regression that correlates spectra and analytic determinations of 240 different lignocellulosic raw materials of *Arundo donax* clones and *Miscanthus* species (stems, leaves or the whole biomass) grown under different agronomic practices and growing seasons. Following a first scan run, spectra of present species under salinity and fertilization treatments were also used for further calibration development in the Ucal software. The same biomass samples were analytically determined in triplicate according to the Van Soest et al. (1991) method for structural carbohydrate and ADL by using a raw fiber extractor (FIWE 6, VELP Scientifica Srl, Usmate, Italy), the Kjeldahl method for proteins (Distillation unit B-324, Büchi Italia Srl), and the ASTM E1755-01 standard for ash.

The statistical methods used in the calibration, for both quantitative and qualitative analysis, are the multiple linear regression of derivative absorbance method and multivariate Partial Least Squares (PLS). The PLS is a technique that decomposes the spectrum in a quantitative way by exploiting the correlation between the spectra data and the constituent concentrations. Before developing the final calibration pretreatments varied were applied, such as the validation cross groups (CV Groups), set at 4, the outlier limits, namely the *t-student* and the global distance, set at 2.5 and 5.0, respectively, and the expansion multiplier, set at 3.

At this point the software processes a new regression that correlates spectra and analytic determinations. The predictive ability of the calibration equations was assessed by the standard error and the coefficient of determination in calibration (SEC and R2C, respectively), and the standard error and the coefficient of determination in cross-validation (SECV and R2CV, respectively).

As both SEC and R2C parameters neglect the variance of the regression coefficients the SECV and R2CV were used to indicate the average prediction error and the proportion of the variance of the dependent variable explained by the regression. These parameters indicate the efficiency of the predictive model and provide an idea of its accuracy.

In addition, the residual predictive deviation (RPD), i.e., the ratio of the standard deviation of the analyzed character and the standard error of the cross-validation, was also taken into account to estimate the efficiency of the calibration.

The new calibration was then used to obtain model robustness from NIRs for cellulose, hemicellulose, ADL, proteins and ash.

Statistical Analysis

Biomass yield data were subjected to the three-way analysis of variance (ANOVA) according to the experimental layout, with species, salinity and fertilization as fixed factors (CoStat, version 6.0). The leaf area index and the instantaneous WUE through the growing season were analysed by a three-way ANOVA using repeated measurements in time, where the growing season represents the within-factor, and the species, salinity and fertilization the between-factor (SPSS, PAWS Statistics 18). When data failed the Mauchly's sphericity test, the univariate results were adjusted by using the Greenhouse-Geisser Epsilon and the Huynh-Feldt Epsilon correction factors. When univariate results satisfied sphericity tests for within subject effects, the F-values and associated P-values for between subject effects were tested. Differences between means were evaluated for significance using the Student-Newman-Keuls (S.N.K.) test at 95% confidence level.

Results

Experiment 1

Morphological and physiological traits

The ANOVA showed a significant effect ($P \leq 0,05$) of species, time of measurement, salinity, and the interaction of species x salinity, salinity x fertilizer and species x salinity x fertilizer on the number of green leaves. Fertilization on interaction of species x time during the measurement and of species x fertilization were not significant (Table 2). The mean separation of main effects revealed a significant difference between species and between salinity, but not between fertilization treatments (Table 3).

Table 2. ANOVA for green leaves using repeated measures on time for main effects (species, salinity and fertilization) and interactions. Mean separation of main effects according to Tukey Method and 95.0% confidence level.

Source	DF	Adj MS	F	P
Species(S)	2	103.483	146.91	0.000
Time(T)	6	45.038	63.94	0.000
S x T	14	1.178	1.67	0.079
Salinity(SL)	2	202.340	287.26	0.000
Fertilier(F)	2	0.244	0.35	0.708
S x SL	4	69.431	98.57	0.000
S x F	4	1.703	2.42	0.051
SL x F	4	10.895	15.47	0.000
S x SL x F	8	7.711	10.95	0.000
Error	144	0.704		

Irrespective of soil salinity and fertilization, the three species showed a decreasing trend as the growing seasons moved forward. In giant reed there were no differences in the control between fertilization treatments, as well as in the S_1 although the number of green leaves was lower than the control.

Surprisingly, in the S_2, *Arundo* under F_2 had as much leaves as the control, however there was a clear effect of the fertilization which reduced the number of green leaves as it was reduced. In *Miscanthus* no differences were observed between fertilization treatment within soil salinity. The S_1 showed green leaves less than the S_0 and the S_2 treatments, which had a similar number of green leaves. *Saccharum* showed a lower number of green leaves as compared with the other species.

Table 3. Mean separation of main effects for green leaves according to Tukey Method and 95.0% confidence level.

Species	***Green leaves (n.)***
Arundo	6.1a
Miscanthus	3.6c
Saccharum	5.3b
Salinity	
S0	6.9a
S1	4.7b
S2	3.4c
Fertilization	
F0	5.1a
F1	4.9a
F2	5.0a

The salinity effect proportionally reduced the number of green leaves as it was increased.

As for the stem height (cm) the ANOVA showed a significant effect ($P \leq 0.05$) of main effects, as well as of the first and second order interactions (Table 4). The mean separation of main effects revealed a significant difference between species and between salinity, but not between fertilization treatments.

Table 4. ANOVA for stem height using repeated measures on time for main effects (species, salinity and fertilization) and interactions. Mean separation of main effects according to Tukey Method and 95.0% confidence level.

Source	DF	Adj MS	F	P
Species (S)	2	24147.4	502.36	0.000
Time (T)	6	1064.1	22.14	0.000
S x T	14	242.4	5.04	0.000
Salinity (SL)	2	35532.3	739.21	0.000
Fertilier (F)	2	469.6	9.77	0.000
S x SL	4	9002.9	187.30	0.000
S x F	4	280.0	5.82	0.000
SL x F	4	1838.8	38.25	0.000
S x SL x F	8	679.2	14.13	0.000
Error	144	48.1		

Across the average of treatment, investigated species showed an increasing trend as the growing seasons moved forward (Figure 5). *Miscanthus* was more responsive to salinity stress as it showed the shorter stem height overall. *Saccharum* was the highest under the S_0, however under the salinity stress giant reed kept the highest stem height. A clear fertilization effect was observed only in giant reed under S_2 condition.

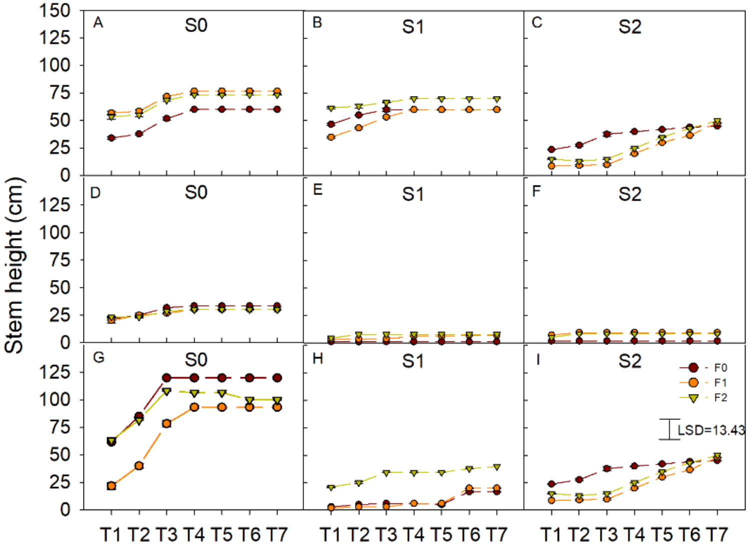

Figure 5. Plant height (cm) of *Arundo donax* (A-C), *Miscanthus* x *giganteus* (D-F) and *Saccharum spontaneum* (G-I) under different salinity levels (S_0, S_1, S_2), and NPK fetilization (F_0, F_1, F_2). Significant LSD interaction of "species x salinity x fertilization" at $P \leq 0.05$ (13.43).

The ANOVA showed a significant effect of measurement time, species and salinity levels, but neither fertilization nor the first order interactions were statistically significant for the leaf area index (Table 5). The second order interaction was marginally significant per $P \leq 0.05$.

The leaf area index (LAI) of the investigated species under salinity and fertilization treatments showed an upward trend followed by a decline as the growing seasons moved forward. *Miscanthus*, followed by *Saccharum* showed the lowest LAI overall, which strongly decreased as the salinity increased, particularly in *Miscanthus*. *Arundo*, on the other hand, seems to be less responsive to soil salinity, as the LAI showed a similar trend between salinity treatments. The fertilization did not show appreciable differences between species and salinity levels (Figure 6).

Table 5. Repeated measure ANOVA for within (time) and between-subject effects (species, salinity, fertilization) (F) on leaf area index (LAI).

Source	**DF**	**Adj MS**	**F**	**P**
Species (S)	2	550.36	239.37	0.000
Time (T)	6	50.38	21.91	0.000
S x T	14	30.27	13.17	0.000
Salinity (SL)	2	10.45	4.55	0.012
Fertilization (F)	2	3.59	1.56	0.213
S x SL	4	4.85	2.11	0.082
S x F	4	2.84	1.23	0.299
SL x F	4	5.49	2.39	0.079
S x SL x F	8	9.53	4.15	0.058
Error	144	2.30		

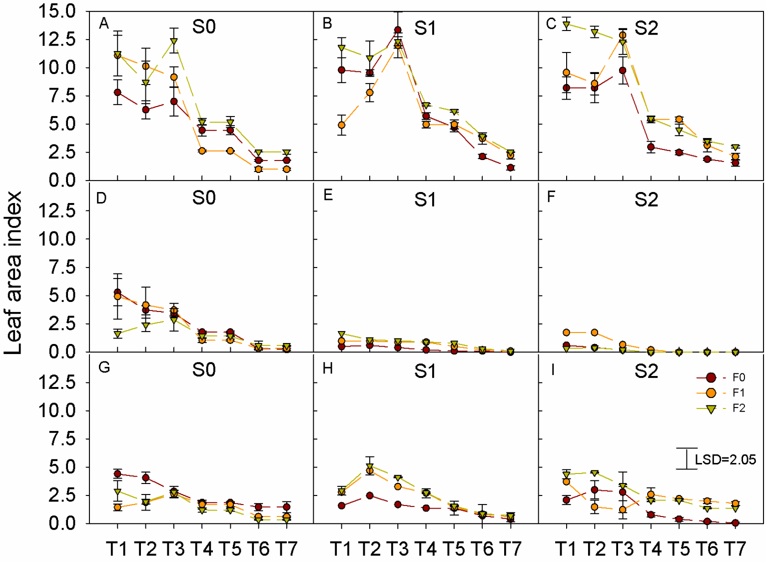

Figure 6. Leaf area index of *Arundo donax* (A-C), *Miscanthus* x *giganteus* (D-F) and *Saccharum spontaneum* (G-I) under different salinity levels (S_0, S_1, S_2), and NPK fetilization (F_0, F_1, F_2). Significant LSD interaction of “species x salinity x fertilization” at $P \leq 0.05$ (2.05).

The ANOVA showed a significant effect of time of measurement, species, salinity and fertilization on instantaneous water use efficiency (iWUE). The species x salinity, salinity x fertilization and species x salinity x fertilization interactions were also significant (Table 6).

Table 6. Repeated measure ANOVA for within (time) and between-subject effects (species, salinity, fertilization) (F) on instantaneous water use efficiency (iWUE)

Source	**DF**	**Adj MS**	**F**	**P**
Species (S)	2	11.25	28.12	0.000
Time (T)	5	35.04	87.59	0.000
S x T	10	0.92	2.31	0.016
Salinity (SL)	2	9.95	24.87	0.000
Fertilier (F)	2	3.19	7.99	0.001
S x SL	4	18.46	46.15	0.000
S x F	4	0.71	1.78	0.138
SL x F	4	4.37	10.92	0.000
S x SL x F	8	4.09	10.23	0.000
Error	121	0.40		

The iWUE of the investigated species under salinity and fertilization treatments showed a fluctuating trend around the mean throughout the growing season (Figure 7). Although the different photosynthetic pathway (C3 for *Arundo* and C4 for both *Saccharum* and *Miscanthus*), the iWUE, which represents the net photosynthesis over the transpiration rate, was similar among species. The mean separation showed that *Miscanthus* and *Arundo* were significantly higher than *Saccharum* across salinity and fertilization, that S_2 was significantly higher than S_1 and S_0 across species and fertilizations, and that F_0 and F_1 were significantly higher than F_2 across species and salinity levels.

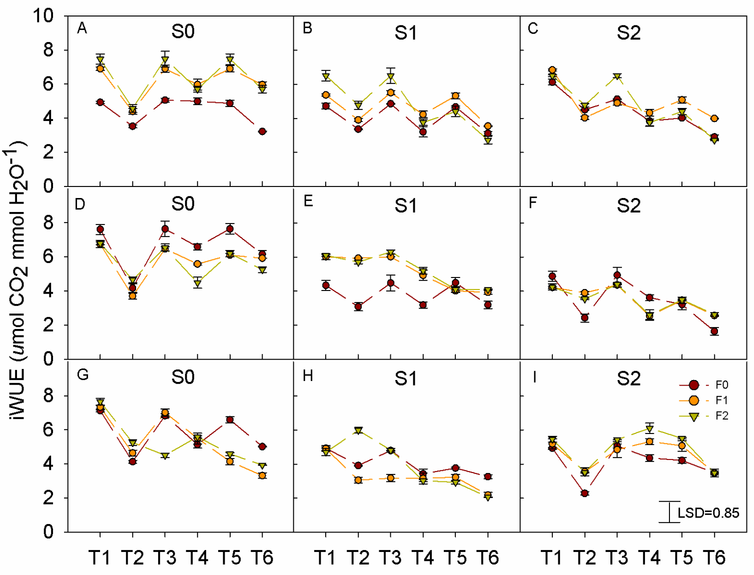

Figure 7. Instantaneous water use efficiency of *Arundo donax* (A-C), *Miscanthus* x *giganteus* (D-F) and *Saccharum spontaneum* (G-I) under different salinity levels (S_0, S_1, S_2), and NPK fetilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at $P \leq 0.05$ (0.85).

Productive traits

Biomass production at harvest is the most important trait to evaluate crop performance under stressful conditions. The ANOVA showed a significant effect of fixed factors and the interaction of species x salinity (Table 7).

The other first order, as well as the second order interactions were not statistically significant.

Table 7. ANOVA for main effects and interactions on biomass production.

Source	DF	Adj MS	F	P
Species (S)	2	1704.2	69.61	0.000
Salinity (SL)	2	1461.6	59.71	0.000
Fertilier (F)	2	233.83	9.55	0.003
S x SL	4	166.47	6.80	0.002
S x F	4	26.77	1.09	0.369
SL x F	4	12.67	0.517	0.723
S x SL x F	8	45.99	1.87	0.083
Error	52	24.48		

Figure 8 showed that biomass production was the significantly highest in *Saccharum* S_0-F_2 (40.6 g plant^{-1}) and the significantly lowest in *Miscanthus* S_1-F_1, *Miscanthus* S_2-F_1, *Miscanthus* S_1-F_0 and *Miscanthus* S_2-F_0 (averaged 2.54 g plant^{-1}). However, *Saccharum* S_0-F_1, *Arundo* S_0-F_1, *Saccharum* S_0-F_0, *Arundo* S_0-F_2 and *Arundo* S_1-F_2 were not statistically different than the highest yielding combination (averaged 29.35 g plant^{-1}).

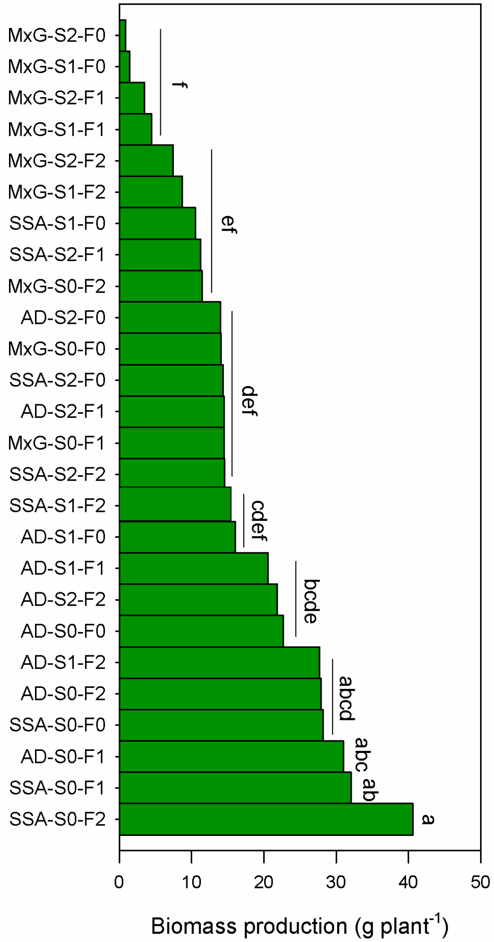

Figure 8. Biomass yield (g plant^{-1}) in combination of experimental factors, namely species (*Arundo donax* - AD, *Miscanthus* x *giganteus* - MxG, *Saccharum spontaneum* - SSA), salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Different letters indicate statistical significance according to the SNK test at $P \leq 0.05$.

The biomass production between the salinity control (S_0) and the medium salinity level (S_1), across fertilizations, reduced by 21% in *Arundo*, 59% in *Saccharum* and 63% in *Miscanthus*. The reduction between S_0 and the highest salinity level (S_2) was 38% in *Arundo*, 60% in *Saccharum* and 70% in *Miscanthus*. *Saccharum* between S_1 and S_2 reduced by only 0.5%, while *Arundo* and *Miscanthus* by 21% and 19%, respectively.

In the salinity control (S_0), the highest fertilization (F_2) improved the biomass production in *Saccharum* by 21% and 30% as compared with the medium (F_1) and unfertilized treatment (F_0). In *Arundo* and *Miscanthus* such improvement was observed only between F_2 and F_0 (18% and 2%, respectively), while between F_2 and F_1 a reduction was registered (-11% and -27% for *Arundo* and *Miscanthus*, respectively). In intermediate salinity level (S_1), *Arundo* under F_2 improved by 25% and 42% as compared with the F_1 and F_0. Such improvement was of 6% and 31% in *Saccharum* and 49% and 93% in *Miscanthus*. In the most stress salinity condition (S_2), the F_2 improved of 33% and 36% the biomass production of *Arundo* as compared with F_1 and F_0, of 53% and 94% in *Miscanthus*, and of 1.7% in *Saccharum* between F_2 and F_0. *Saccharum* reduced by 29% the biomass production of F_2 as compared with F_1 in S_2.

The mean separation of the main factors showed a higher biomass production and not statistically different for *Arundo* and *Saccharum*, and the lowest for *Miscanthus*. Biomass dry weight was significantly highest in S_0, followed by S_1 and by S_2, while among fertilization, the biomass dry weight was the highest in F_2 and the lowest but not different in F_1 and F_0 (Figure 9).

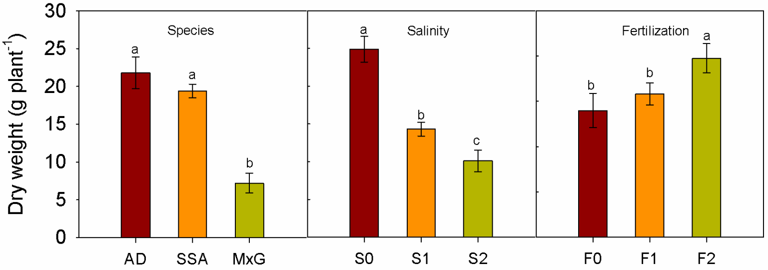

Figure 9. Mean significance of species, salinity and fertilization main effects. Different letters indicate statistical significance according to the SNK test at $P \leq 0.05$.

Biomass quality

The table 8 shows the ANOVA for hemicellulose content on leaves of perennial grasses under salinity and fertilization levels. The three main effects were highly significant, as well as the interactions, except the third order one. The mean separation of main effect showed that leaves of *Miscanthus* and *Saccharum* had a significantly higher hemicellulose content than *Arundo*. The soil salinity increase lead to a linear decrease of this component on leaves, while increasing the fertilization showed the opposite trend (Table 9).

Table 8. ANOVA for main effects and interactions on hemicellulose content on leaf.

Source	DF	Adj MS	F	P
Species (S)	2	176.14	371.85	0.000
Salinity (SL)	2	55.28	116.70	0.000
Fertilier (F)	2	2.94	6.22	0.004
S x SL	4	3.11	6.58	0.000
S x F	4	2.21	4.67	0.003
SL x F	4	1.16	3.41	0.015
S x SL x F	8	0.85	1.80	0.097
Error	54	0.47		

Table 9. Mean separation of main effects for hemicellulose content on leaf according to Tukey Method and 95.0% confidence level.

Species	***H_leaf***
Arundo	28.92b
Miscanthus	33.47a
Saccharum	33.21a
Salinity	
S0	33.34a
S1	31.80b
S2	30.48c
Fertilization	
F0	31.49b
F1	32.07a
F2	32.05a

The hemicellulose content on stems was significant for the species and salinity main effect, while did not for the fertilization (Table 10). There were significant interactions, like species x fertilization, salinity x fertilization, and the species x salinity x fertilization. The mean separation of main effect showed a significantly higher hemicellulose content on the stems of *Arundo* and *Saccharum* and the lowest in *Miscanthus*. The salinity also in this case led to a decrease of this component on the stems, while the fertilization did not make substantial changes (Table 11).

Table 10. ANOVA for main effects and interactions on hemicellulose content on stems.

Source	DF	Adj MS	F	P
Species (S)	2	94.27	313.22	0.000
Salinity (SL)	2	38.70	128.61	0.000
Fertilier (F)	2	0.261	0.87	0.426
S x SL	4	0.683	2.27	0.073
S x F	4	5.01	16.67	0.000
SL x F	4	3.20	10.64	0.000
S x SL x F	8	1.12	3.73	0.002
Error	54	0.30		

Table 11. Mean separation of main effects for hemicellulose content on stems according to Tukey Method and 95.0% confidence level.

Species	***H_stem***
Arundo	29.31a
Miscanthus	27.01b
Saccharum	29.15a
Salinity	
S0	29.34a
S1	28.18b
S2	26.94c
Fertilization	
F0	28.04a
F1	28.20a
F2	28.22a

The cellulose content on leaves was significant for only species and salinity (Table 12). The ANOVA, however, showed also a significant effect of salinity x fertilization interaction. Among species, *Saccharum* had the significantly highest content of cellulose on leaves, followed by *Msicanthus* and *Arundo* with the lowest. Increasing the salinity, decreased the cellulose content on leaves, while the fertilization had no effect (Table 13).

Table 12. ANOVA for main effects and interactions on cellulose content on leaves

Source	DF	Adj MS	F	P
Species (S)	2	215.06	412.19	0.000
Salinity (SL)	2	45.37	86.96	0.000
Fertilier (F)	2	0.24	0.47	0.629
S x SL	4	0.84	1.62	0.183
S x F	4	3.43	6.56	0.000
SL x F	4	0.241	0.46	0.763
S x SL x F	8	0.312	0.60	0.755
Error	54	0.522		

Table 13. Mean separation of main effects for cellulose content on leaves according to Tukey Method and 95.0% confidence level.

Species	***C_leaf***
Arundo	24.72c
Miscanthus	28.79b
Saccharum	30.14a
Salinity	
S0	29.20a
S1	27.83b
S2	26.61c
Fertilization	
F0	27.99a
F1	27.81a
F2	27.85a

Similarly, the ANOVA for the cellulose content on stems showed a significant effect of species, salinity and the interaction between these two main factors (Table 14).

Table 14. ANOVA for main effects and interactions on cellulose content on stems.

Source	DF	Adj MS	F	P
Species (S)	2	36.64	63.30	0.000
Salinity (SL)	2	10.69	18.48	0.000
Fertilier (F)	2	5.40	9.33	0.000
S x SL	4	0.19	0.34	0.847
S x F	4	2.29	3.96	0.007
SL x F	4	0.37	0.65	0.628
S x SL x F	8	0.56	0.97	0.466
Error	54	0.57		

Among species, *Arundo* showed the significantly highest cellulose content on stems, followed by *Miscanthus* and by *Saccharum*. Also, in this case increasing the salinity level decreased significantly the cellulose content on stems (Table 15).

Table 15. Mean separation of main effects for cellulose content on stems according to Tukey Method and 95.0% confidence level.

Species	***C_stem***
Arundo	31.43a
Miscanthus	30.35b
Saccharum	29.10c
Salinity	
S0	30.92a
S1	30.30b
S2	29.66c
Fertilization	
F0	30.73a
F1	30.32ab
F2	29.83b

The figure 10 and 11 show the interaction of the main factors on the biomass parameters analyzed on the leaves and stems of *Arundo*, *Miscanthus* and *Saccharum*.
Hemicellulose content in *Arundo* was consistently distributed on stems and leaves, while both *Miscanthus* and *Saccharum* showed a higher hemicellulose content on the leaves than the stems (Figure 10).

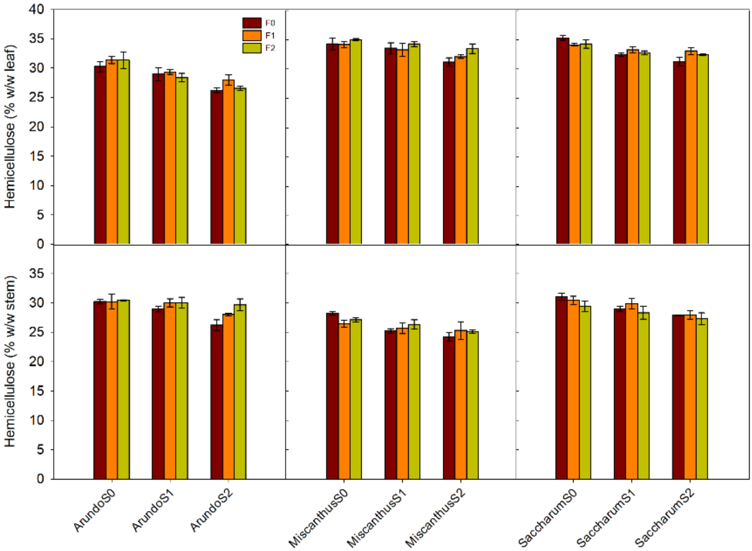

Figure 10. Hemicellulose content on leaves (above) and stems (below) of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fetilization (F_0, F_1, F_2).

On the cellulose front, *Saccharum* had a similar content between stems and leaves, while *Arundo* had a higher content on stems than leaves and *Miscanthus* a higher content on leaves than stems (Figure 11).

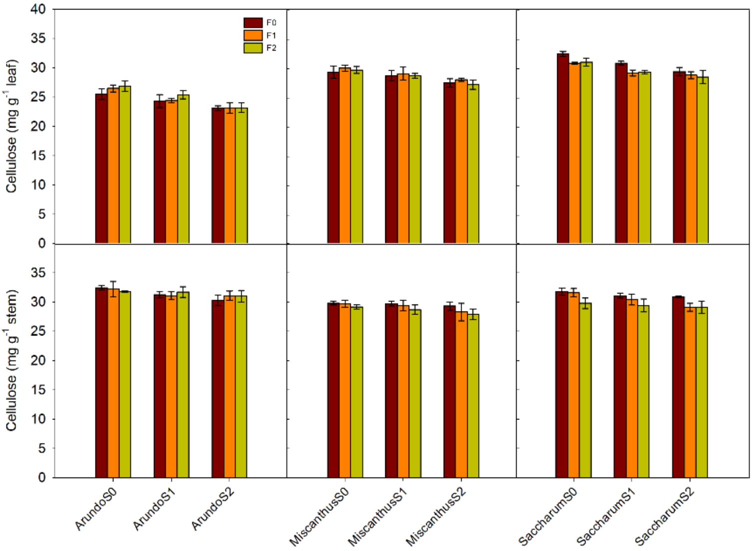

Figure 11. Cellulose content on leaves (above) and stems (below) of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2).

Experiment 2

Soil salinity

Electrical conductivity (EC) is the most common measurement of soil salinity and is indicative of the ability of a water solution to carry out an electric current. It is commonly expressed in units of deciSiemens per meter (dS m^{-1}). By agricultural standards, The JRC study indicated that soils are classified affected by salinity if the topsoil electrical conductivity is equal or higher than 3.2 dS/m.

Soil salinity level (dS m^{-1}) has been detected on May 27 2020 at the beginning of the irrigation treatment with desired NaCl in three different levels (S_0, S_1 and S_2), adding the solution approximately once a week per pot.

After one month of irrigation treatment with tap water or NaCl, EC increased significantly in S_1 and S_2 treatments, as well as on the S_0. The fertilization at the highest level (F_2) increased the EC in all species as compared with the medium level fertilization (F_1) and no fertilization (F_0). The EC reached between 5.8 and 7.75 dS m^{-1} in *Arundo* S_0 at F_0 and F_2 respectively. In *Miscanthus* S_0, the EC was 4.7 and 5.9 dS m^{-1} in F_0 and F_2, while in *Saccharum* S_0 the EC was 4.8 and 6.1 dS m^{-1} in the same fertilization order (Figure 12).

The S1 treatment after irrigation with NaCl reached at the final detection time the value of 18.2-19.4 dS m^{-1} in *Arundo*, 18.4-18.7 dS m^{-1} in *Miscanthus* and 18.3-18.6 dS m^{-1} in *Saccharum* at F_0 and F_2, respectively. The highest salinity level (S_2) reached 21.6-22.9 dS m^{-1} in *Arundo*, 21.2-23.1 dS m^{-1} in *Miscanthus* and 23.1-25.8 dS m^{-1} in *Saccharum* at F_0 and F_2, respectively.

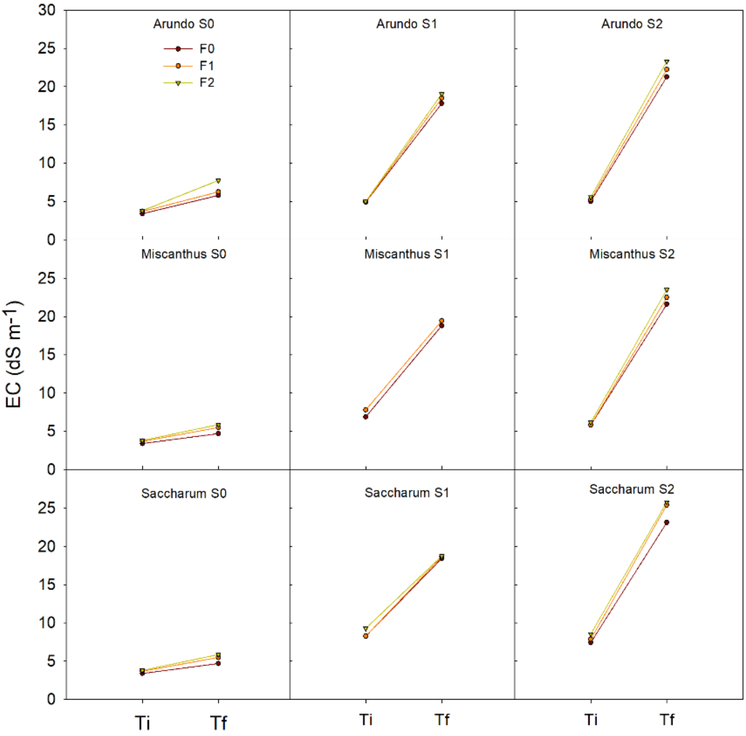

Figure 12. Soil electrical conductivity (EC, dS m^{-1}) at the beginning (Ti) and final period of NaCl application (Tf) in *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2).

Plant biometric characters

The ANOVA for stem height shows a significant effect of all main effects and interactions, except the tree-order interaction at $P \leq 0.05$ (Table 16).

Table 16. ANOVA for main effects and interactions on stem height at harvest.

Source	DF	Adj MS	F	P
Species (S)	2	11375.4	1756.5	0.000
Salinity (SL)	2	2590.1	399.9	0.000
Fertilier (F)	2	278.8	43.05	0.000
S x SL	4	180.4	27.86	0.000
S x F	4	56.6	8.74	0.000
SL x F	4	26.3	4.06	0.006
S x SL x F	8	18.6	2.87	0.010
Error	54	6.50		

The mean separation of main effects species indicated that *Saccharum* was the tallest, followed by *Arundo* with *Miscanthus* the shortest one. There was a salinity gradient, with higher soil salinity level decreasing stem height. Fertilization followed also a gradient, the higher the dose the higher the plant stems (Table 17).

Table 17. Mean separation of main effects for stems height according to Tukey Method and 95.0% confidence level.

Species	***height***
Arundo	64.81b
Miscanthus	37.60c
Saccharum	77.77a
Salinity	
S0	70.45a
S1	58.66b
S2	51.04c
Fertilization	
F0	56.55c
F1	60.64b
F2	62.88a

The interaction of effect on plant stem height is shown in figure 13. It is clear that *Saccharum* was the tallest species, both under fertilization and salinity treatments. Overall, the shortest plant was *Miscanthus* in S_2 and F_0 condition (31 cm) and the tallest was *Saccharum* under S_0 and F_1 and F_2 conditions (94 cm averaged).

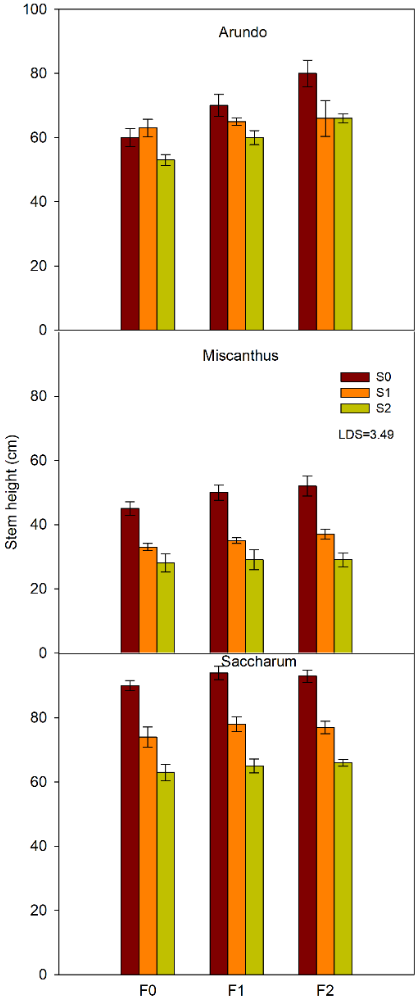

Figure 13. Stem height of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at $P \leq 0.05$ (3.49).

The leaf area index (LAI) at harvest was significantly affected by main effects (species, salinity and fertilization) and by species x salinity interaction alone (Table 18).

Table 18. ANOVA for main effects and interactions on leaf area index at harvest.

Source	DF	Adj MS	F	P
Species (S)	2	36.90	132.65	0.000
Salinity (SL)	2	18.31	65.84	0.000
Fertilier (F)	2	16.67	59.95	0.000
S x SL	4	1.40	5.04	0.002
S x F	4	0.30	1.09	0.369
SL x F	4	0.09	0.35	0.845
S x SL x F	8	0.14	0.50	0.848
Error	54	0.27		

The mean separation showed a significantly higher LAI value in *Arundo*, followed by *Miscanthus* and by *Saccharum* (Table 19). Increasing the salinity level decreased significantly the LAI in all species, while fertilization increased the value of LAI.

Table 19. Mean separation of main effects for on leaf area index at harvest according to Tukey Method and 95.0% confidence level.

Species	***LAI***
Arundo	5.82a
Miscanthus	4.01b
Saccharum	3.64c
Salinity	
S0	5.19a
S1	4.68b
S2	3.58c
Fertilization	
F0	3.62c
F1	4.56b
F2	5.11a

The interaction of main effects on LAI is shown in figure 14. With a least significant difference among treatments of 0.71, *Arundo* was clearly the species with the highest LAI values in all conditions. Among salinity treatments, the S_0 and S_1 were not that different in *Arundo* and *Miscanthus* and the LAI difference became evident with the S_2 treatment. In *Saccharum*, the three salinity treatments did not show appreciable differences.

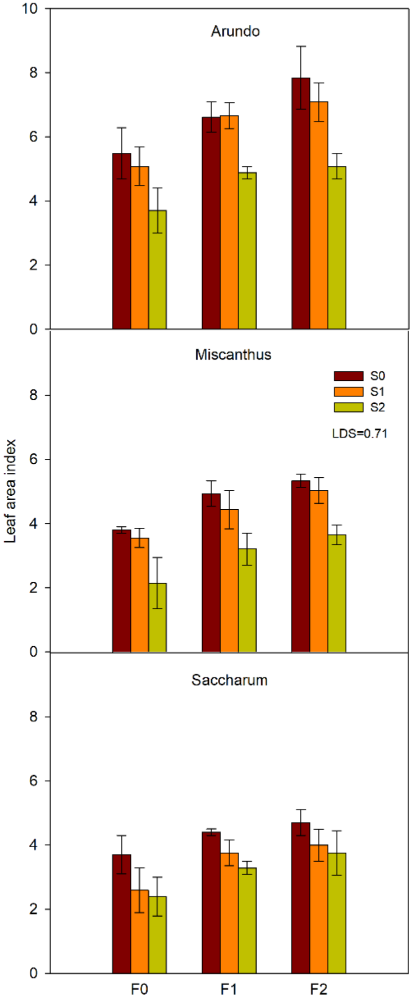

Figure 14. Leaf area index *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at $P \leq 0.05$ (0.71).

Biomass yield

The biomass at harvest was separated in its main aboveground components (leaves and stems) and root. The ANOVA for leaf dry weight was significant for all main effects and interactions, except for the species x fertilization interaction (Table 20).

Table 20. ANOVA for main effects and interactions on leaf weight.

Source	DF	Adj MS	F	P
Species (S)	2	40.71	146.34	0.000
Salinity (SL)	2	37.93	136.36	0.000
Fertilier (F)	2	15.07	54.19	0.000
S x SL	4	12.48	44.88	0.002
S x F	4	0.92	3.33	0.016
SL x F	4	5.93	21.35	0.000
S x SL x F	8	7.02	25.25	0.000
Error	54	0.27		

The mean separation of main effects showed that *Miscanthus* had the highest leaf dry weight at harvest, followed by *Arundo* and by *Saccharum* (Table 21). The salinity effect leads to a decrease at the highest level (S_2) however, no differences were observed between S_0 and S_1. The fertilization was effective at all levels, with the F_0 showing the lowest leaf dry weight and the F_2 the highest.

Table 21. Mean separation of main effects for on leaf weight at harvest according to Tukey Method and 95.0% confidence level.

Species	***Leaf***
Arundo	10.98b
Miscanthus	12.06a
Saccharum	9.62c
Salinity	
S0	11.62a
S1	11.52a
S2	9.52b
Fertilization	
F0	10.14b
F1	10.89b
F2	11.63a

The ANOVA for stem dry weight was significant for all main effects and interactions (Table 22).

Table 22. ANOVA for main effects and interactions on stem weight.

Source	**DF**	**Adj MS**	**F**	**P**
Species (S)	2	91.34	163.52	0.000
Salinity (SL)	2	304.14	544.48	0.000
Fertilier (F)	2	23.50	42.07	0.000
S x SL	4	15.37	27.51	0.000
S x F	4	11.42	20.45	0.000
SL x F	4	1.59	2.86	0.032
S x SL x F	8	6.87	12.30	0.000
Error	54	2.43		

The mean separation of main effects showed that *Arundo* had the highest stem dry weight at harvest, followed by *Saccharum* and by *Miscanthus* (Table 23). The salinity effect leads to a decrease increasing its level, with the S_2 the lowest and the S0 the highest. Also, the fertilization increased stem dry weight by increasing the level (F_0 the lowest and the F_2 the highest).

Table 23. Mean separation of main effects for on stem weight at harvest according to Tukey Method and 95.0% confidence level.

Species	***Stem***
Arundo	14.85a
Miscanthus	11.91c
Saccharum	13.30b
Salinity	
S0	16.83a
S1	13.09b
S2	10.14c
Fertilization	
F0	12.48c
F1	13.25b
F2	14.34a

The ANOVA for root dry weight was significant for species and fertilization main effects but did not for the salinity (Table 24). Only the species by salinity interaction was significant for root dry weight, while the other interactions, both of second and third order were not.

Table 24. ANOVA for main effects and interactions on root weight.

Source	DF	Adj MS	F	P
Species (S)	2	2229.68	72.39	0.000
Salinity (SL)	2	32.60	1.06	0.354
Fertilier (F)	2	409.88	13.31	0.000
S x SL	4	118.86	3.86	0.008
S x F	4	37.87	1.23	0.309
SL x F	4	21.39	0.69	0.599
S x SL x F	8	12.94	0.42	0.904
Error	54	30.80		

The mean separation showed the highest root dry weight in *Msiacnthus* and the lowest in *Saccharum* (Table 25). The fertilization, at the highest dose only (F_2) increased root dry weight as compared with F_0 and F_1, while no salinity effect increased this trait.

Table 25. Mean separation of main effects for on root weight at harvest according to Tukey Method and 95.0% confidence level.

Species	***Root***
Arundo	27.69b
Miscanthus	36.68a
Saccharum	18.50c
Salinity	
S0	27.69a
S1	28.64a
S2	26.49a
Fertilization	
F0	23.88b
F1	27.34b
F2	31.65a

Figure 15 shows the biomass weight and partitioning of all species and treatments tested. Overall, *Miscanthus* and *Arundo* did not show appreciable differences in the whole biomass weight (root + leaf + stem), however, the different biomass partition suggests that *Miscanthus* keeps stable the belowground biomass as the soil became more saline and reduces the aboveground weight, while *Arundo* and *Saccharum* tends to reduce all biomass components proportionally to the salinity levels. In all cases, fertilization seems to mitigate the salinity levels in all species, particularly at the highest doses.

The least significant difference of species x salinity x fertilization interaction was quite small for leaf dry weight (0.71), intermediate for stem dry weight (2.10) and quite high for root dry weight (7.59). The overall lowest leaf dry weight was observed in *Saccharum* at S_2 and F_0 conditions (5.49 g), while the overall highest in *Miscanthus* S_1F_2, S_0F_2 and S_1F_1 (14.87 g averaged). The stem dry weight was the lowest, among all treatments, in *Miscanthus* S_2F_0 (6.22 g) and the highest in *Arundo* S_0F_2 (20.1 g). The root dry weight was the lowest in *Saccharum* S_1F_0 (13.18 g), which however was not different than *Saccharum* S_1F_1, S_1F_2, S_2F_0, S_2F_1, S_0F_1 and S_2F_2 (17.94 g on average). The highest root dry weight was in *Miscanthus* S_2F_2 (44.78 g).

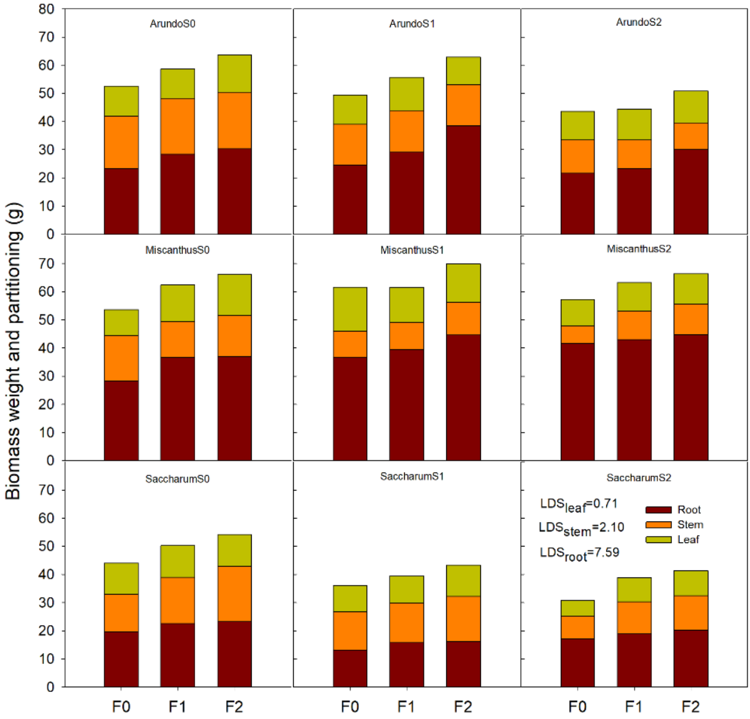

Figure 15. Biomass weight and partitioning of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at $P \leq 0.05$ for leaf (0.71), stem (2.10) and root (7.59).

Plant physiology

The ANOVA for the stomatal conductance (gs) shows a significant effect of all main effects and interactions at $P \leq 0.05$ (Table 26).

Table 26. ANOVA for main effects and interactions on stomatal conductance.

Source	**DF**	**Adj MS**	**F**	**P**
Species (S)	2	0.0315	65670.9	0.000
Salinity (SL)	2	0.0053	11063.8	0.000
Fertilier (F)	2	0.0005	121.58	0.000
S x SL	4	0.0006	1415.55	0.000
S x F	4	0.00003	60.79	0.000
SL x F	4	0.00006	138.95	0.000
S x SL x F	8	0.00005	104.21	0.000
Error	54	0.00002		

Mean separation was conducted for main effects (Table 27). Among species, the C3 *Arundo* had the significantly highest gs, while the two C4, *Saccharum* and *Miscanthus* did not differ. Among salinity treatments, the lower the soil salinity the highest the gs across the average of species and fertilization main effects. The increase in fertilization amount led to a significant increase of gs across the species and salinity.

Table 27. Mean separation of main effects for stomatal conductance (gs) according to Tukey Method and 95.0% confidence level.

Species	***gs***
Arundo	0.092a
Miscanthus	0.033b
Saccharum	0.033b
Salinity	
S0	0.066a
S1	0.054b
S2	0.038c
Fertilization	
F0	0.051c
F1	0.055b
F2	0.058a

The interaction of main effects on gs is shown in figure 16. With a least significant difference among treatments of 0.01, *Arundo* was clearly the species with the highest gs values in all conditions, even those at the highest stress level and fertilization amount.

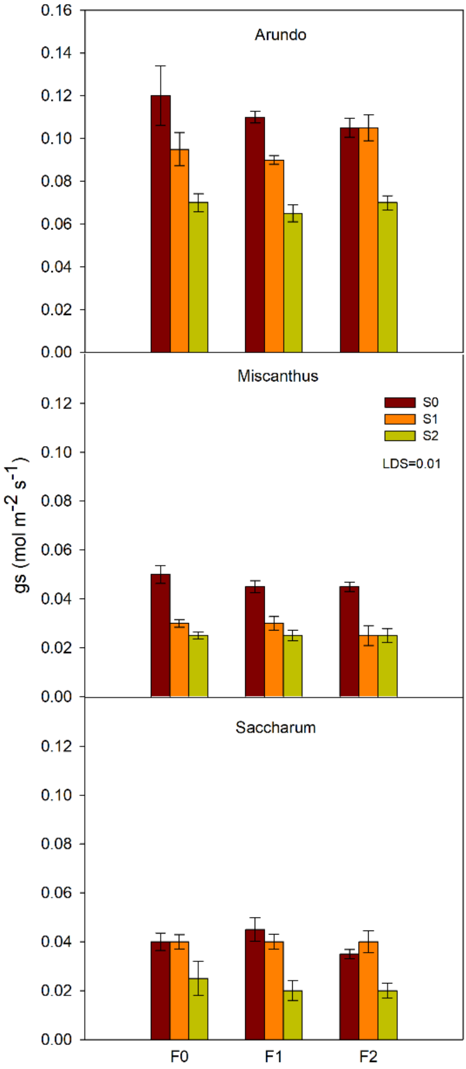

Figure 16. Stomatal conductance (gs, mol m^{-2} s^{-1}) of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at P≤0.05 (0.01).

The ANOVA for instantaneous water use efficiency (iWUE) is shown in table 28. There was a significant effect of species and salinity but not of fertilization. Among interactions, only the species x salinity was significant.

Table 28. ANOVA for main effects and interactions on instantaneous water use efficiency.

Source	**DF**	**Adj MS**	**F**	**P**
Species (S)	2	96.94	158.94	0.000
Salinity (SL)	2	15.90	26.08	0.000
Fertilier (F)	2	1.43	2.36	0.104
S x SL	4	11.13	18.25	0.000
S x F	4	0.95	1.57	0.197
SL x F	4	0.99	1.63	0.179
S x SL x F	8	1.16	1.92	0.076
Error	54	0.61		

The mean separation of main effects supported the photosynthetic metabolism of C4 species on iWUE, with *Saccharum* and *Miscanthus*, although statistically different, having the highest iWUE. *Arundo*, with its C3 had the lowest. The salinity stress led to a decrease in iWUE across species and fertilization treatments, however, both S_1 and S_2 were not different among them. The fertilization did not show any statistical effect, although it increased the iWUE.

Table 29. Mean separation of main effects for instantaneous water use efficiency (iWUE) according to Tukey Method and 95.0% confidence level.

Species	***iWUE***
Arundo	2.40c
Miscanthus	5.02b
Saccharum	6.08a
Salinity	
S0	5.38a
S1	4.07b
S2	4.03b
Fertilization	
F0	4.25a
F1	4.54a
F2	4.70a

The interaction of main effects on iWUE is shown in figure 17. The two C4 species, and particularly *Saccharum* had highest iWUE among species, both on unstressed and salinity stress conditions. Within species, the salinity treatment seems to not reduce this trait, due mainly to the concurrent reduction of net photosynthesis and transpiration rate. *Miscanthus* had the second highest iWUE, while *Arundo*, a C3 crop showed the lowest. In this case the fertilization did not mitigate the salinity stress, although it slightly increased this trait.

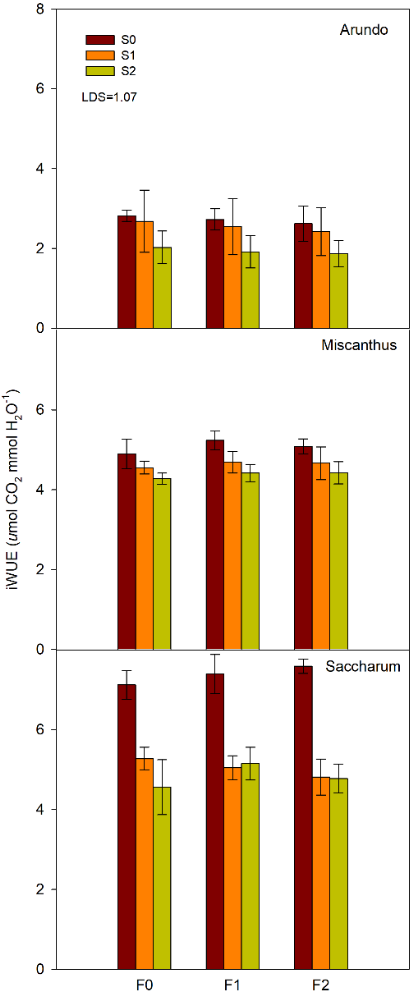

Figure 17. Instantaneous water use efficiency (iWUE, µmol CO_2 mmol H_2O^{-1}) of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at $P \leq 0.05$ (1.07).

The ANOVA for the maximum quantum efficiency of photosystem II (Fv/Fm) was significant for fertilization and salinity main effects, while species was not significant on this physiological trait (Table 30). However, the species by salinity interaction was highly significant but the other second and third order did not.

Table 30. ANOVA for main effects and interactions on maximum quantum efficiency of photosystem II.

Source	**DF**	**Adj MS**	**F**	**P**
Species (S)	2	0.001	0.85	0.433
Salinity (SL)	2	0.45	231.8	0.000
Fertilier (F)	2	0.10	5.39	0.007
S x SL	4	0.12	6.46	0.000
S x F	4	0.001	0.57	0.686
SL x F	4	0.0006	0.33	0.854
S x SL x F	8	0.0015	0.77	0.634
Error	54	0.002		

The table 31 shows the mean comparison of main effects. Species were quite similar across the salinity and fertilization, while salinity main effect, across fertilization and species decreased from 0.753 at unstressed condition (S_0) to 0.494 under the most stress salinity level. Fertilization increased slightly, but significantly the Fv/Fm, which was 0.607 at F_0 and 0.646 at F_2. F_1 condition was not statistically different than F_0 and F_2.

Table 31. Mean separation of main effects for maximum quantum efficiency of photosystem II (Fv/Fm) according to Tukey Method and 95.0% confidence level.

Species	***Fv/Fm***
Arundo	0.633a
Miscanthus	0.618a
Saccharum	0.621a
Salinity	
S0	0.753a
S1	0.625b
S2	0.494c
Fertilization	
F0	0.607b
F1	0.620ab
F2	0.646a

The interaction of main effects on the Fv/Fm shows a more consistent response in *Arundo*, where there were slight differences between the salinity levels moving from the S_0 to S_1 and from S_1 to S_2 in all fertilization levels (Figure 18). In *Miscanthus*, on the other hand, the response to salinity level increase was much stronger, and the maximum quantum efficiency of photosystem II consistently reduced of 0.2 from S_0 to S_1 and from S_1 to S_2 condition. In *Saccharum* the response of Fv/Fm was intermediate between the two species and the fertilization seems to mitigate someway the salinity stress mainly at the highest level.

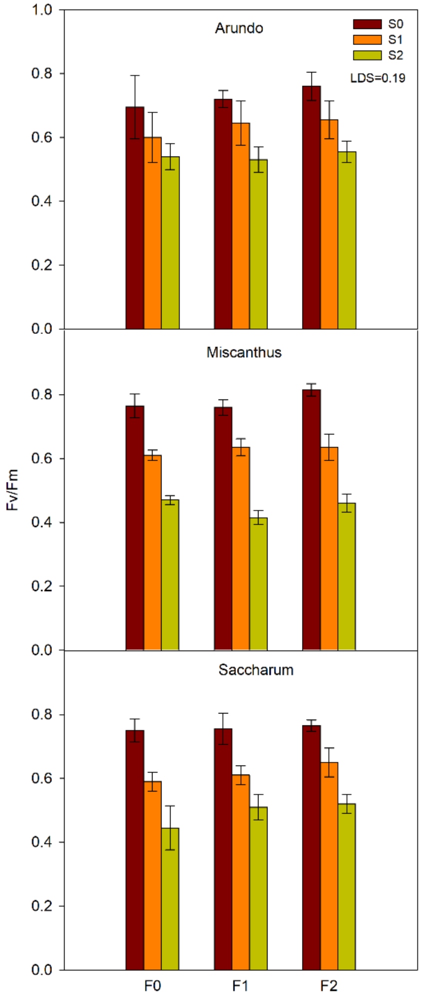

Figure 18. Maximum quantum efficiency of photosystem II (Fv/Fm) of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at $P \leq 0.05$ (0.19).

Biomass quality

The main effects and interaction for biomass quality, namely protein, hemicellulose, cellulose, ADL and ash on leaves are shown in table 34. The ANOVA showed significant species effect for all quality parameters, the salinity was significant for all except the cellulose content, while the fertilization for hemicellulose and ADL content only. The specie x salinity interaction was significant for the hemicellulose and ADL, while the interactions species x fertilization and salinity x fertilization were significant for cellulose and protein, respectively.

Table 34. ANOVA for main effects and interactions for biomass quality (protein, hemicellulose, cellulose, ADL and ash) on leaves.

Source	**DF**	**Prot.**	**Hemic.**	**Cellul.**	**ADL**	**Ash**
				Adj MS		
S	2	665.5^{***}	209.2^{***}	293.3^{***}	10.4^{***}	120.2^{***}
SL	2	4.06^{*}	31.41^{**}	8.06	37.2^{***}	22.5^{***}
F	2	32.6^{**}	2.44	4.80	0.32	4.04^{*}
S x SL	4	2.75	16.98^{*}	1.75	3.49^{**}	0.75
S x F	4	0.88	1.99	12.04^{*}	0.42	0.33
SL x F	4	3.76^{*}	5.21	0.99	0.26	0.38
SxSLxF	8	1.84	6.30	2.51	0.56	0.69
Error	54	1.14	4.94	3.66	0.38	0.96

The mean separation of main effects for biomass quality on leaves is shown in table 35. The protein content was significantly higher in *Arundo* than *Saccharum* with *Miscanthus* showing the lowest content. The salinity decreased protein content however, it was not different between medium and highest level. Contrarily, increasing the fertilization increased the protein content on leaves.

Table 35. Mean separation of main effects for biomass quality on leaves (mg g^{-1}) according to Tukey Method and 95.0% confidence level.

Species	***Protein***	***Hemic.***	***Cellulose***	***ADL***	***Ash***
Arundo	14.95a	34.79c	26.62b	10.85a	7.96a
Miscanthus	5.55c	36.37b	26.22b	10.63a	5.17b
Saccharum	7.46b	40.20a	32.12a	9.68b	3.97c
Salinity					
S0	9.77a	36.02b	27.69a	9.06c	7.32a
S1	9.06b	38.17a	28.66a	10.80b	6.28b
S2	9.15ab	37.18ab	28.61a	11.30a	5.49c
Fertilization					
F0	8.06b	37.26a	28.56a	10.49a	5.92b
F1	9.86a	37.33a	28.58a	10.38a	6.53ab
F2	10.05a	36.78a	27.83a	10.29a	6.64a

The hemicellulose content was the highest in *Saccharum* leaves followed by *Miscanthus* and by *Arundo*. Increasing the salinity level increased the hemicellulose, while no effect was observed for the fertilization on this biomass quality trait. The cellulose was the highest in *Saccharum* and not different in *Miscanthus* and *Arundo* leaves. Salinity and fertilization did not affect this trait. The ADL was the highest in *Miscanthus* and *Arundo* and the lowest in *Saccharum*. The salinity increased ADL content but the fertilization did not. Finally, the ash content was the highest in *Arundo*, intermediate in *Miscanthus* and the lowest in *Saccharum*. The increase in salinity levels decreased the ash content, while increasing the fertilization proportionally raised the ash.

The interaction of main effects for biomass quality on leaves and the calculated least significant difference for each biomass component is shown in Figure 19.

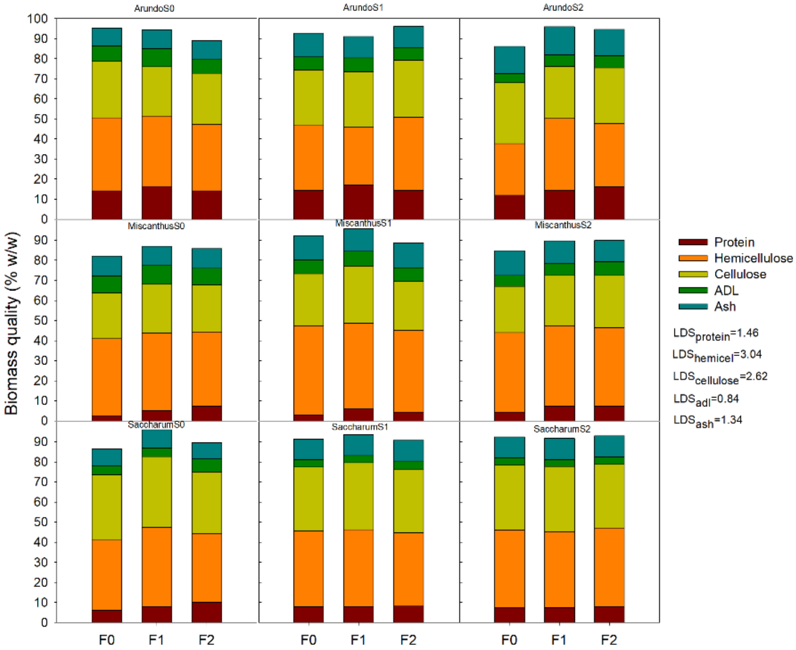

Figure 19. Biomass quality on leaves (%w/w) of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at P≤0.05.

The main effects and interaction for biomass quality, namely protein, hemicellulose, cellulose, ADL and ash on stems are shown in table 36. The ANOVA showed significant species effect for all quality parameters, except for the cellulose content. The salinity was significant only for ash content, while the fertilization for protein and ash. The specie x salinity interaction was significant all quality component, the interactions species x fertilization for protein, the interaction salinity x fertilization for protein, ADL and ash and three order interaction for ash.

Table 36. ANOVA for main effects and interactions for biomass quality (protein, hemicellulose, cellulose, ADL and ash) on stems.

Source	DF	Prot	Hemic.	Cell	ADL	Ash
		Adj MS				
S	2	55.7^{***}	131.0^{***}	9.52	18.8^{**}	3.1^{**}
SL	2	0.11	0.93	2.84	0.61	4.4^{***}
F	2	15.3^{**}	6.71	0.31	0.21	7.0^{***}
S x SL	4	3.18^{**}	9.90^{*}	12.42^{*}	9.37^{**}	22.7^{**}
S x F	4	0.51	8.93^{*}	2.70	0.80	0.25
SL x F	4	2.29^{*}	5.52	1.74	1.66^{*}	1.23^{*}
SxSLxF	8	1.97^{*}	2.08	1.38	0.79	0.79^{*}
Error	54	0.51	3.12	3.44	0.44	0.36

The mean separation of main effects for biomass quality on stems is shown in table 37. As observed for leaves, the protein content was significantly higher in *Arundo* than *Saccharum* with *Miscanthus* showing the lowest content. The salinity did not affect this trait, while the fertilization increased the protein content also on stems.

Table 37. Mean separation of main effects for biomass quality on stems (mg g^{-1}) according to Tukey Method and 95.0% confidence level.

Species	***Protein***	***Hemic.***	***Cellulose***	***ADL***	***Ash***
Arundo	6.97a	34.75b	32.53a	11.46a	4.18a
Miscanthus	4.24c	38.12a	31.48a	11.16a	3.25b
Saccharum	4.85b	33.98b	32.49a	9.89b	3.66b
Salinity					
S0	5.31a	35.44a	32.46a	10.71a	3.43b
S1	5.32a	35.81a	31.82a	10.80a	3.74b
S2	5.42a	35.60a	32.21a	11.01a	4.21a
Fertilization					
F0	4.48b	35.83a	32.29a	10.75a	3.22b
F1	5.72a	35.97a	32.09a	10.83a	3.98a
F2	5.85a	35.05a	32.12a	10.93a	4.19a

The hemicellulose content was the highest in *Miscanthus* stems followed by *Saccharum* and *Arundo*. Salinity and fertilization levels did not affect this trait on stems. The cellulose content on stems was not statistically different among species, salinity and fertilization treatments. The ADL content was the highest in *Miscanthus* and *Arundo* and the lowest in *Saccharum*. The salinity and the fertilization were not effective in ADL content. The ash content was the highest in Arundo, and the lowest but not statistically different in Miscanthus and Saccharum. The increase in salinity and fertilization levels increased the ash content.

The interaction of main effects for biomass quality on leaves and the calculated least significant difference for each biomass component is shown in Figure 20.

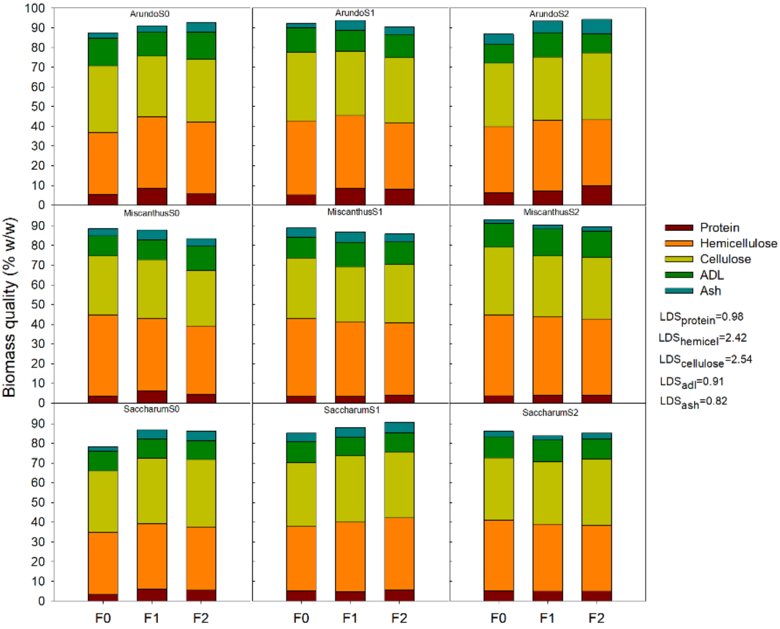

Figure 20. Biomass quality on stems (%w/w) of *Arundo donax*, *Miscanthus* x *giganteus* and *Saccharum spontaneum* under different salinity levels (S_0, S_1, S_2), and NPK fertilization (F_0, F_1, F_2). Significant LSD interaction of species x salinity x fertilization at P≤0.05

Conclusions

Growing bioenergy crops on marginal lands might in part mitigate the land abandonment due to degradation and unproductivity, reduce the land use changes and provide the raw material for a bioeconomy development.

However, crops with excellent adaption strategies to growing limiting conditions are necessary to produce sufficient and profitable biomass under certain environments dominated by biophysical constraints. The JRC study indicated that soils are classified affected by salinity if the topsoil electrical conductivity is equal or higher than 3.2 dS m^{-1}.

In Experiment 1, the study highlights the high tolerance to salinity of the investigated perennial grasses, as the salinity levels employed were, respectively 2.8-fold (S_1) and 5.6-fold (S_2) higher than the threshold of 3.2 dS m^{-1}.

While morphological and physiological traits throughout the experimental period were someway affected, there was a not clear trend with the treatments imposed to the crops, although main effects were statistically significant.

On the other hand, biomass productivity at harvest is the most important trait to evaluate crop performance under stressful conditions. The mean separation of the main factors showed a higher biomass production for *Arundo* and *Saccharum* than for *Miscanthus*. Salinity followed a gradient, decreasing as the salinity level increased: the biomass production between the salinity control (S_0) and the medium salinity level (S_1), across fertilizations, reduced by 21% in *Arundo*, 59% in *Saccharum* and 63% in *Miscanthus*. The reduction between S_0 and the highest salinity level (S_2) was 38% in *Arundo*, 60% in *Saccharum* and 70% in *Miscanthus*.

Fertilization had a beneficial effect in the different salinity levels and crops. As expected, the highest fertilization clearly improved the biomass production in all crops under not saline environments. In a salinity of 9 dS m^{-1} (S_1), the highest fertilization (F_2) improved biomass production in *Arundo* by 25% and 42%, by 6% and 31% in *Saccharum* and by 49% and 93% in *Miscanthus* as compared with the F_1 and F_0, respectively. Under a salinity of 18 dS m^{-1} (S_2), the F_2 improved by 33% and 36% the biomass production in *Arundo* and by 53% and 94% in *Miscanthus* as compared with F_1 and F_0. The trend was not clear for *Saccharum*, which showed an increase of 1.7% between F_2 and F_0, but a decrease of 29% in F_2 as compared with F_1.

The biomass quality in Experiment 1 was carried out for hemicellulose and cellulose content on stems and leaves. The soil salinity increase lead to a linear decrease of hemicellulose content both on leaves and stems, while increasing the fertilization increased hemicellulose on leaves but not in stems. Among species, *Miscanthus* and *Saccharum* had a significantly higher hemicellulose content than *Arundo* in leaves, while *Saccharum* and *Arundo* on stems. *Arundo* showed also the significantly highest cellulose content on stems, followed by *Miscanthus* and by *Saccharum*. Also, in this case, increasing the salinity level decreased significantly the cellulose content on stems. Increasing fertilization decreased cellulose content on stems but was ineffective on leaves.

In Experiment 2 a different substrate and propagation material was used. The soil salinity at the end of the experiment reached, across the average of species and fertilization treatments, nearly 20 dS m^{-1} in S_1 and 25 dS m^{-1} in S_2 due to the effect of medium brackish irrigation water.

Main findings suggested that there was an inverse response of morphological (stem height and leaf area index) and productive traits (stems, leaves and root dry weight) to increasing salinity levels. On the other hand, increasing fertilization levels with NPK increased the main morphological and productive traits mitigating, in part, the salinity stress. Among species, *Saccharum* was the tallest, *Arundo* had the highest LAI, *Miscanthus* the highest leaf and root dry weight, while *Arundo* the highest stem dry weight.

As expected, the C3 *Arundo* showed the significantly highest stomatal conductance, which lead to a higher transpiration rate among species. The two C4, *Saccharum* and *Miscanthus,* although did not differ their stomatal conductance showed a significantly different instantaneous water use efficiency, with *Saccharum* having the highest overall, however, both were more efficient than *Arundo*. The salinity stress reduced both stomatal conductance and the instantaneous water use efficiency, while the fertilization increased both parameters, but the significant effect was observed only for the stomatal conductance. On the other hand, the maximum efficiency of photosystem II was quite similar among the C3 and the C4 crops. The fertilization increased slightly this trait, while the salinity stress had a strong, depressing effect on the maximum efficiency of photosystem II. It is worth to mention that Arundo seems to be less affected to harsher conditions, reducing this trait to a lesser extent as compared to the other species.

The biomass quality was affected to a different extent in relation to the treatments, species and plant part. Generally, leaves had a higher amount of protein and ash than stems, while stems had a higher amount of cellulose and ADL. The fertilization increased both protein and ash in leaves and stems, but the structural compounds (i.e., hemicellulose, cellulose and ADL) were less affected. The increase in salinity decreased the protein and increased the hemicellulose on leaves, while increased the ash on stems. The other biomass compounds were not significantly modified by salinity. Among species, *Arundo* had the highest protein and ash, both on stems and leaves, the hemicellulose was the highest in *Saccharum* leaves and in *Miscanthus* stems, the cellulose was the highest in *Saccharum* and *Miscanthus* leaves, while the three species had a smilar cellulose amount on stems. The ADL was the highest in *Miscanthus* and *Arundo* and the lowest in *Saccharum*, both stems and leaves.

According to the results of both experiment 1 and 2, the soil salinity at the end of the experiment reached nearly 20 dS m^{-1} in S_1 and 25 dS m^{-1} in S_2 indicating a strong adaption of these species under harsh prone environments subjected by to salinity and or the irrigation with brackish water

However, further work is necessary to confirm these results on different type of soils, the mechanism of resilience of these crops to salinity and other possible mitigation strategies.

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