THE RESILIENCE OF *Typha domingensis* Pers. TO NUTRIENT-DEPLETED WATER IN A FLOATING BIOMASS PRODUCTION SYSTEM

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ABSTRACT: An aquatic system of biomass production based on *Typha domingensis* Pers. (cattail) for biofuels and bio-products was developed in order to combine the production of amylaceous (rhizomes) and lignocellulosic biomass (shoots) for biofuels and biobased materials with the improvement of water quality and the reduction of GHG emissions. The proof-of-concept was implemented in the framework of the LIFE Biomass C+ Project, co-funded by the European Commission. This article reports on implementation activities carried out in Spain in the first stage of the Project and their results. Cattails were artificially grown as floating aquatic plants with the help of ad-hoc designed supports on irrigation ponds at El Arenal (province of Ávila, Spain) in order to build green floating filters. Biomass was fully harvested at the end of the growth cycle. Biomass production, biomass partitioning and energy and chemical properties were determined. Eutrophication was not detected. *T. domingensis* exhibited great resilience to nutrient-depleted conditions and was able to produce a significant amount of biomass.
Keywords: Biomass, feedstock, innovative concepts, climate change mitigation.

1 INTRODUCTION

The LIFE Programme is a funding instrument of the European Union for the environment and climate action, being one of the priority areas of the climate action, the mitigation of climate change [1]. In the call of 2016, the project entitled ‘Low-cost, carbon positive bioethanol production with innovative green floating filters in multiple water bodies’ (acronym: LIFE Biomass C+) was granted under the climate change mitigation area, for the period 2017-2021. The Project aims at the production of sustainable biofuels from Green Floating Filters (GFFs) of *Typha domingensis* Pers. (cattail), grown on multiple water bodies. Implementation actions (actions ‘C’) encompass installation of GFFs in Greece and Spain (C1), biomass processing and bioconversion to ethanol (C2), and replication and future applications (C3). In addition to the development of a strategy for climate change mitigation through the production of sustainable biofuels, other expected environmental benefits of the Project are the improvement of water quality and the avoidance of emissions associated with Land Use Change [2].

This article reports on C1 implementation activities carried out in Spain in the first stage of the LIFE Biomass C+ Project, from plant propagation to GFFs’ biomass production. Biomass properties and estimates of nutrients removal and C capture –activities under Action C3 – are reported as well.

2 MATERIAL AND METHODS

2.1 Steps in the implementation process

In the framework of Action C1 of the LIFE Biomass C+ Project, the installment of GFFs in Spain involved three organizations: the Agro-Energy Group of the Technical University of Madrid (GA-UPM), the Irrigators’ Community of El Arenal (COMRA), and VOLTERRA Ecosystems S.L. (VOLTERRA), in addition to the coordination of different actors –farmers, technicians and researchers- engaged with the network created for the Project. Following technical specifications of the Project, the site for GFFs installment in Spain (Action C1) was the municipality of El Arenal (province of Ávila). It is worth noting that, due to the characteristics of the site (see 2.2) personal consent of farmers was requested from the side of COMRA in order to cede use of irrigation ponds in their properties.

The implementation process consisted of the following steps: production of cattail plants (plant propagation), establishment of GFFs, monitoring and evaluation of GFFs’ biomass production. Biomass properties and estimates of nutrients removal and C capture –activities under Action C3 of the Project– were assessed as well. Site description and methodology for each step are described below.

2.2 Production of cattail plants

Cattails propagation can be done by rhizomes (vegetative or asexual propagation) or by seeds (sexual propagation). The former is quicker in growth than the latter but generally it does not allow species identification. Due to the fact that cattail tolerance to eutrophication is different from one species to another, species identification was considered key for the LIFE Biomass C+ project; therefore, plant propagation was made by seeds.

Spadices of wild cattail stands were collected in October 2016 and taken to laboratory for species identification by means of botanical keys [3]. Spadices identified as *Typha domingensis* were taken apart and left at room temperature for natural release of cattail fruits (achenes). Afterwards, seeds were separated by gravity and conveniently stored at 4ºC.

The production of cattail plantlets was carried out at the facilities of the Agro-Energy Group, Technical University of Madrid (Spain), coordinates 40º 26' 36'' N and 3º 44' 18'' W; altitude 650 m.a.s.l. The climate is continental-Mediterranean, with 15.0ºC mean temperature and 421 mm annual rainfall [4]. In order to have cattail plantlets ready for GFFs establishment in spring, a semi-cylindrical greenhouse (polyethylene cover) was made available for seedbeds.

Cell seed trays (52.5 x 33.5 x 7.8 cm, 96 cells) were filled with a seedbed substrate and placed inside shallow ponds containing 3-4 cm of water height at the GA-UPM nursery. A pinch of seeds was sown by hand on each cell (cattail seeds are very small, about 0.05-0.11 mg mass per seed). Seeds were let to germinate and soon after, seedlings started growing. When they reached a height of about 20-30 cm, they were thinned to one seedling per cell; cell trays were kept wet inside the greenhouse until transportation to El Arenal.

2.3 Site description

El Arenal (Ávila, Spain) is a small village (973 people [5]) on south-facing slopes of Gredos Mountains; longitude 41º 15' 53" N, latitude 5º 5' 14" W, altitude 888 m.a.s.l. The village is named after the stream of the same name, tributary of the Tiétar River (Hydrographic Confederation of Tagus River). Natura 2000 sites of code ES0000184 (Valle del Tiétar-ZEPA) and ES4110115 (Valle del Tiétar) are in the vicinity of El Arenal.

Prior to the start of the implementation actions of the Project, it was checked that cattails were present in the area to prevent the introduction of new species into the region. Literature review showed that *Typha domingensis* and *Typha latifolia* are abundant in the province of Ávila [6]. In addition to the literature review, the area was surveyed in September 2017 for the presence of *T. domingensis*. Large colonies of cattails were found at the sites located at 40º 12' 56" N, 5º 6' 24" W (Arenas de San Pedro municipality) and 40º 15' 13" N, 5º 3' 31" W (El Arenal municipality).

The climate in the study area is classified as 'Upper Mesomediterranean Low Humid' according the Bioclimatic Classification by Rivas-Martínez [7]; Köppen climate category is 'Csb' (mild winter and dry summer). Annual means are: 11.5ºC mean temperature, 1375 mm rainfall, 4.65 $kWh \cdot m^{-2} . day^{-1}$ solar radiation.

The landscape is a mixture of dispersed orchard terraces, forest (*Pinus pinaster, Quercus pyrenaica* and other tree species) and shrub land. Main crops are chestnut, fig and cherry trees. Most farms have small ponds in their properties for crop irrigation in summertime (dry season), which are built on terraces, taking advantage of slopes. Irrigation ponds usually have continuous water flow throughout the year. The flow of influent water in irrigation ponds was measured at the very beginning of the Project (10 September 2017). At that time (end of the summer dry period), when the influent water was expected to be little, the flow was 1.1 $L \cdot min^{-1}$ at the inlet of irrigation ponds.

In order to meet the target area set in the Technical Annex of the Project, four irrigation ponds were made available in 2018 by COMRA associates for the Project. Net size (water surface) of the ponds in this study ranged from 21 to 94 m^2.

2.4 GFF design and establishment

The installment of green floating filters based on helophytes such as cattails requires two basic elements: i) a system of support that allows plants to grow like free-floating plant species, and ii) plants with sizes suitable for the chosen system, capable of forming a floating plant mat.

Ideally, the system of support should be as simple as possible to facilitate scaling up. Supports can be manufactured with several compounds, which entail different characteristics such as biodegradability, resistance, floatability, and price; they can be shaped in the form of sheets, pots, ropes, trays, or nets. Generally modular assembling of sheets sizing 0.5-1 m^2 each results quite versatile and functional. Sheets made up of extruded polystyrene (XPS) and Expanded Polystyrene (EPS) performed well in previous GFFs experiments developed by GA-UPM [8]; however, EPS sheets were up to 50 % cheaper than XPS sheets, depending on the ordered quantity and material density.

Following a price criterion, the material chosen for this work was EPS with a density of 20 $kg \cdot m^{-3}$. Dimensions of each EPS sheet were: 120 x 40 x 5 cm (length, width, and height). Sheets had perforations (holes) to hold plant balls and to allow roots growing freely when submerged in water while shoots could emerge above the surface of EPS sheets (EPSS). Each sheet had seven alternate rows of round holes 4.5 cm in diameter, 8-9 $holes.row^{-1}$. EPSS were provided by VOLTERRA, partner in the LIFE Biomass C+ Project.

Planting was performed from 24 April to 5 May 2018. Cattails in cell trays (see 2.2) were trimmed prior to transportation and fertilized with a foliar fertilizer to stimulate growth. They were taken to El Arenal farms as required. Plants were removed from cells one by one and placed in alternate EPSS holes; on average, 15-16 plants per sheet were used. Empty holes were expected to be colonized by new shoots growing from rhizomes of planted cattail plants, during the growth cycle. After planting, EPSS were placed on the water surface of each pond.

2.5 Monitoring

Records of daily temperatures and precipitation were compiled from the closest weather station in order to relate plant growth to environmental conditions. Data were retrieved from the weather station 'Fuente-Refugio El Hornillo', situated about 6 km away from El Arenal, at longitude 40º 16' 6" N; latitude 5º 7' 22" W (time zone 30, X=319502.7; Y= 4459698.4; Z= 1145) [9].

Ponds were sampled for water quality. Water analyses included pH (pH-meter Crison MM 40, reference temperature RT=25ºC), Electrical Conductivity (EC) (portable conductivity meter MM 40 RT=25ºC), Chemical Oxygen Demand (COD) (dichromate reflux method) and nitrogen ($N\text{-}NH_4$, $N\text{-}NO_3$; Kjeldahl method); standard methods [10] were adapted to GA-UPM laboratory means.

2.6 Evaluation of GFF biomass production

The evaluation of biomass production was performed at the dormancy cattail stage, when plant growth was ended, and the growth cycle was over. Initial date for biomass evaluation was 28 November 2018; the weather did not allow working on the ponds in December. Field works ended on 22 January 2019.

All ponds were sampled for biomass production. Ten percent of EPS sheets were pulled out from each pond; the number of shoots (number of holes with shoots in a sheet) was counted. The emergent biomass (shoots) was cut down, collected and placed into a plastic bag conveniently identified. The submerged biomass was left to drain for about 2 hours and then it was placed into a plastic bag conveniently identified. Afterwards, plastic bags were sealed and taken to laboratory. Biomass was not washed in order to simulate industrial biomass management.

GFFs' aerial biomass production was determined as follows. The emergent biomass of each EPSS was weighed as a whole (Fresh weight, FW) and the value was referred to EPSS area. Then, one sample of about 200 g FW was taken, weighed and oven-dried (80ºC) until constant weight to calculate the dry matter content (% DM). The value of % DM was extrapolated in order to calculate the yield in dry biomass of each GFF (kg per square meter of EPSS).

EPSS submerged biomass was weighed in fresh (FW) and the fresh mass was referred to EPSS area, like it was made for the emergent biomass. In order to quantify the production of rhizomes, fresh samples of whole submerged biomass were taken at random; rhizomes were separated from roots by hand. FW and DW were determined following a similar procedure to the one described in the previous paragraph.

Samples of dried biomass were ground in a blade mill using a 1.2 mm mesh screen; sub-samples were re-ground in an IKA A10 analytical mill to <1 mm. All samples were placed in zip-lock plastic bags until analysis.

2.7 Biomass characterization

Ground samples of the submerged biomass taken from each pond were analyzed in triplicate for carbohydrates content. The contents in free reducing sugars (water-soluble carbohydrates) (FRS), total reducing sugars (hydrolyzed water extract) (TRS) and starch (hydrolysis of the residue from water extraction) (St) were determined by using Nelson-Somogyi reagent [11]. Results were expressed in percentage (dry mass basis).

Fibers content of the aerial biomass collected from each pond was assessed. Neutral detergent acid fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined in triplicate in a Foss FiberTec 1020, based on [12]. Cellulose content was calculated as ADF minus ADL.

Energy properties and nutrient content of three biomass fractions, shoots, roots and rhizomes, were determined in triplicate for each pond. Procedure for volatile matter and ash content followed UNE-CEN 15148 and UNE-CENT/TS-14775 norms. Fixed carbon was calculated as the difference between 100 and the sum of volatile matter and ash. Higher Heating Value (HHV moisture free) was determined in a calorimeter Leco AC-350. Biomass content in total nitrogen, phosphorus and potassium followed procedures described in [13].

3 RESULTS

3.1 Meteorology

Daily mean, maximum and minimum temperatures recorded from 1 April 2018 to 1 February 2019 are depicted in Figure 1.

April and May with 9.0ºC and 12.6 mean temperature, and -0.2ºC and 2.2ºC minimum temperature respectively, were not favorable for plant growth, either November with 7.2ºC and 1.0ºC mean and minimum temperature, respectively. April and November were very rainy, with 144.6 and 246.8 mm precipitation, respectively. May and October with 12.6 and 12.4ºC mean temperature and <10ºC minimum temperature, were also cold for cattail growth. On the contrary, temperatures were mild from June to September, when the monthly mean temperature attained 17.7, 20.4, 23.9 and 20.3ºC. Heavy rains (>30 mm·day^{-1}) were recorded during the autumn (11/10, 14/10, 30/10, 5/11, 12/11) (Figure 2). Assuming 10ºC as the minimum temperature for cattail growth, the actual duration of the growth season in this trial would be 4 months, i.e. from June to September.

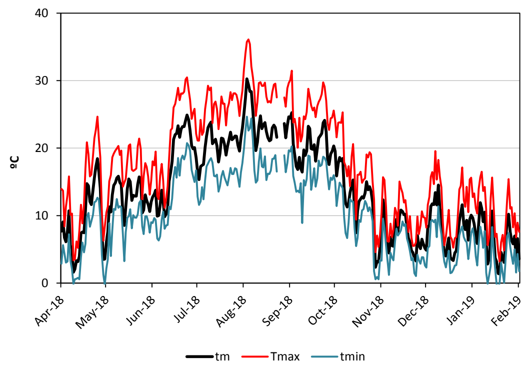

Figure 1: Mean, maximum and minimum temperatures (tm, Tmax, t_{min}).

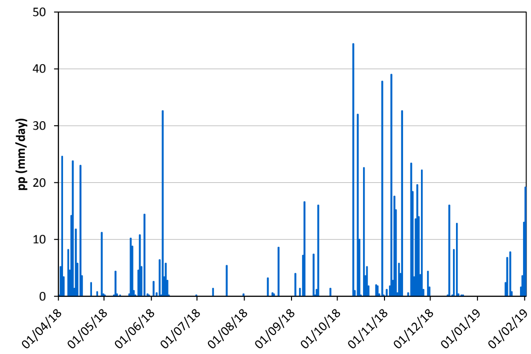

Figure 2: Daily precipitation (pp).

3.2 Water quality

Results of water analyses are shown in Figure 3. Mean values over the sampling period were: pH 6.6; electrical conductivity, 86 µS·cm^{-1}; chemical oxygen demand (COD), 48 mg O_2·L^{-1}; N-NH_4, 4 ppm; N-NO_3, 12 ppm; and P, 0.8 ppm. Variability was observed from pond to pond and also from date to date; in general terms, values were indicative of non-eutrophic water.

Values of single measurements in pond samples ranged from 5.9 to 7.7 pH, from 42 to 172 µS·cm^{-1} E.C. from 0 to 170 mg O_2·L^{-1} COD, from 0 to 30 mg N-NH_4 L^{-1}, from 0 (=undetectable) to 41 mg N-NO_3 L^{-1}, and from 0 to 3.7 mg P·L^{-1}, respectively. As a reference, the range of values reported for urban wastewaters is given next: pH 6-9; EC, 1000-2000 µS·cm^{-1}; COD, 300-700 mg O_2·L^{-1}; total N, 20-85 mg·L^{-1}; total P, 4-15 mg·L^{-1} [14]. According to the classification by [15], typical values for a medium level of contamination are: pH 6.9; COD, 500 mg O_2·L^{-1}; total N, 50 mg·L^{-1}; total P, 7 mg·L^{-1}. In Europe, Directive 91/271/EC [16] establishes the requirement of $\leq$125 mg O_2·L^{-1} COD for waste water treatment plant discharges; in case of sensitive areas, there are requirements for N and P, specifically <15 mg·L^{-1} total N (10,000-100,000 equivalent population (e.p.) agglomerations) and 2 mg·L^{-1} total phosphorus (10,000-100,000 e.p.). In El Arenal, total nitrogen only achieved a value >15 mg·L^{-1} on 25/04 (only one sample) and on 23/09 (three samples), which was attributed to agricultural practices. COD only attained values >125 mg O_2·L^{-1} in the sampling of 23/09, a fact that was related to low water height and presence of microalgae.

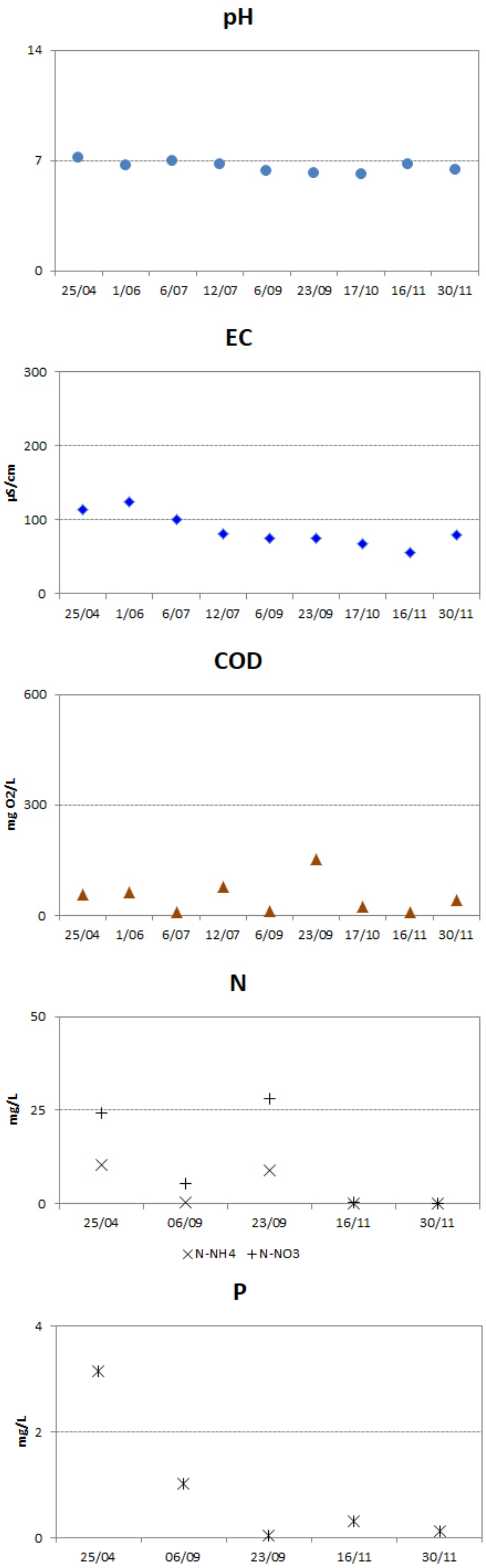

Figure 3: Water characteristics over the season.

3.3 Biomass production

The number of EPSS evaluated for plant growth was 44 (n=44) sampled from 4 irrigation ponds. All plants grown on a sheet (0.5 m^2) were taken as a whole; biomass in a single EPSS represented one sample.

The height of the emergent biomass ranged from 0.76 to 1.24 m, with a mean value of 1.02 m. Plant density increased from 30 plants·m^{-2} at planting date to 37 plants·m^{-2} at harvest time, on average; however, high differences in tillering were recorded among ponds (57 % coefficient of variation).

The dry matter content of the emerged biomass ranged from 13.9 to 37.8 % (24 % coefficient of variation, cv), where the lower values corresponded to EPSS with still green biomass, and the higher values to samplings with senescent or dry biomass. The range observed for the submerged biomass was narrower, from 15.9 to 24.3 % (16 % cv). These values are referred to just-harvested aquatic biomass. One and a half months after harvesting, the dry matter content of the biomass stored at the open air went down to 87 % (w/w). Rhizomes represented 55.0 % (26 % cv) of the submerged biomass (dry weight) (Figure 4).

Total biomass production varied among samplings of a same pond (30-58 % cv) as well as among the ponds (16 % cv). Therefore, variability could be attributed to intrinsic plant characteristics rather than to differences among ponds. Pooled mean was 3.9 kg fresh matter.m^{-2} (↔39 t FM.ha^{-1}) (39 % cv) partitioned into 22.0 % emerged biomass and 78 % submerged biomass (fresh weight basis). Mean dry matter content of whole biomass was 19.5 % (11 % cv). By extrapolation, the yield obtained in this study was equivalent to 7.6 t DM·ha^{-1} after 4-month cattail growth in nutrient-depleted conditions.

The potential of cattails for biomass production has been reported much higher than the mean yield found in this work. In a previous study of *T. domingensis* GFFs, the yield in total biomass attained 8 kg DM·m^{-2} (↔80 t DM ha^{-1}) in conditions of high COD [8]. Mean yield reported by Martín and Fernández attained 13 kg DM·m^{-2} of total biomass [17]. Grosshans estimated 15-20 t DM·ha^{-1} $year^{-1}$ in terms of emergent biomass (‘*dead dry standing material*’, in the words of the author) [18]. Literature shows that cattail growth depends on nutrients availability among other factors. In a study of the effect of five nutrient levels on seven wetland plant species, *T. latifolia* was the highest yielding crop and, also the plant species that showed the best response to increasing nutrient availability. The above-ground biomass increased from 0.7 (lowest nutrient level) to 4.45 kg DM·m^{-2} while the belowground biomass from 1.63 to 4.49 kg DM·m^{-2}, which would represent 89.4 t DM·ha^{-1} for the best nutrient conditions [19]. The increase in total biomass production of *T. angustifolia* (DM) from oligotrophic to hypertrophic nutrient conditions was nearly 400 % [20].

Water in most irrigation ponds of El Arenal could be classified as oligotrophic on the basis of its nutrient content and transparency. *T. domingensis* was able to survive in these conditions and yield a significant amount of biomass. Literature data show that cattails have a high biomass production potential in eutrophic water conditions; results from this study show the resilience of cattails to oligotrophic conditions.

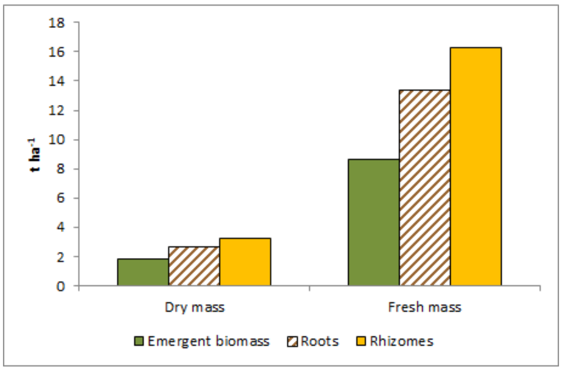

Figure 4: Biomass production.

3.4 Biomass characterization

At harvest, rhizomes contained 26.2 % starch, 7.4 % TRS and 1.9 % FS (dry weight basis) whereas root contents in starch, TRS and FRS were much lower (11.1 %, 0.7 % and 0.9 %, respectively). Biomass partitioning into rhizomes and roots is extremely important for starch yield of the submerged biomass. If the submerged biomass were used as a raw material for 1st generation bioethanol [21], the proportion of rhizomes should be as high as possible. Besides, seasonal changes in the carbohydrates content of cattail organs have been reported in the literature, showing the importance of the harvest date (i.e. the phenological stage of cattails) for starch content. In a study of the starch content in *T. latifolia* organs (natural cattail stand) carried out from November to April, Kausch et al. found 27.4 % starch in rhizomes at the beginning of the year [22].

Fibers in the aerial biomass presented values slightly higher (58.9 % NDF on average, 8.7 % coefficient of variation) than the values reported for cattail green forage by González et al. [23], which suggests that actively-growing shoots have lower proportion of fibers than end-of-season shoots (mostly dry biomass). Interestingly, the cellulose content was very similar, 29.4 % vs 29.8 % [23].

Results of proximate analysis are shown in Figure 5. Mean ash content (weighed mean of all plant organs) was 9.3 %, a value higher than the 4.1 % grass average [24]. The submerged biomass contained 72.3 % volatile matter, 19.1 % fixed carbon and 8.6 % ash on average, and the emerged biomass, 71.9 %, 18.3 % and 9.9 %, respectively. Ash content in the emerged biomass was close to the 10.2 % reported for *T. latifolia* shoots harvested at the end of autumn [25]. However, the range of values reported in the literature for cattail biomass is very wide. In a study carried out in South Carolina (USA), where the spring growth of cattails was dated on 29 March and the senescence of shoots on 1 August, it was found that the ash content of *T. latifolia* live shoots (dead shoots from the previous year were not harvested) decreased from 10.13 % in April to 4.21 % in July [26]. Other study reported values much higher, 12.6 % and 17.0 % ash for shoot and rhizomes of *T. x glauca* in Manitoba (USA), respectively [27]. Different from these works, in our study the biomass was harvested at the winter dormant period. It should be mentioned that high ash values are often related with the presence of inorganic contaminants (introduced ash); this could be the case of this study since biomass was not washed or conditioned.

Energy content of the studied plant fractions was in line with ash results; HHV_0 of the roots (16.0 $MJ·kg^{-1}$) was lower than for shoots (17.5 $MJ.kg^{-1}$) and rhizomes (17.6 $MJ·kg^{-1}$). Calorific value and ash content of the harvest would likely improve if biomass were washed to remove soil and dust particles (introduced ash). Anyway, the obtained values of calorific value are within the range of values reported in the literature for *Typha* spp.; from 16.0 [28] to 19.63 $MJ·kg^{-1}$ [23] for the aboveground biomass, and 15.1 $MJ·kg^{-1}$ for the rhizomes [28]. According to these values, the use of cattail shoots (harvested at the end of the growth cycle, when they are dry) for thermal energy seems a good option, little explored so far [29].

Nutrient content of GFFs' biomass varied among plant organs. At harvest, rhizomes contained more N (2.0 %) and P (0.3 %) than roots and shoots, and shoots contained more K (1.0 %) than rhizomes and shoots. Literature data show that the dynamics of nutrients in cattails is quite complex. Martin & Fernández [17] showed that N and P in cattail biomass peaked in April whereas minimum values were recorded in October-November, i.e. at the dormant period. These authors reported 2.02 % N and 0.33 % P in rhizomes, and 1.46 % N in roots; 1.11 % N and 0.14 % P in shoots. Other authors reported a positive relationship between nutrients in *T. latifolia* biomass and nutrients in the growth medium [19, 30]. Rem et al. reported 2.11 % N, 0.06 % P and 0.98 % K (harvest in September) for the green leaf fraction of cattails grown in a low-nutrient medium [19]. All these values are in line with the values obtained in this study.

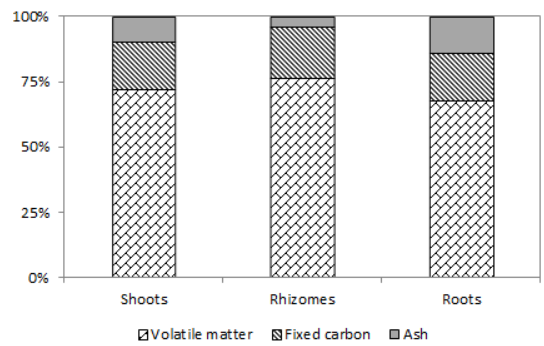

Figure 5: Mean contents in volatile matter, fixed carbon and ash.

Nutrients accumulation in cattail biomass and carbon captured by GFFs were estimated from the results of biomass production, biomass partitioning, biomass content in macronutrients and carbon content of the emerged and submerged biomass.

On average, nutrients accumulation in cattail biomass represented a removal of 12.16 g N, 1.64 g P and 10.1 g K per GFF square meter over the growth cycle. Nearly 90 % of the N and P uptaken by the crop were allocated to the submerged biomass (Figure 6).

The amount of carbon captured by GFFs of *Typha domingensis* grown in nutrient-depleted conditions was estimated at 1.25 kg $CO_2·m^{-2}$, where 70.5 % was allocated to the submerged biomass. These values show that, for optimal N and P removal and C capture, it is advisable to harvest not only the emergent biomass but also the submerged biomass. In principle, GFFs have the advantage over rooted cattail colonies that the submerged biomass is generally easier to pick up.

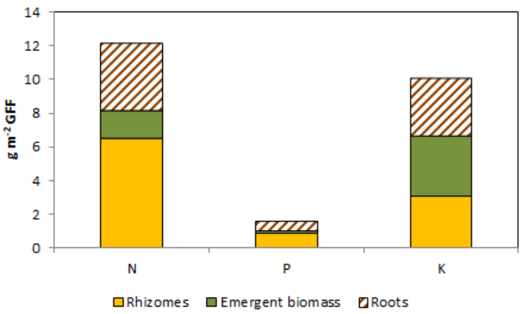

Figure 6: Nutrients in GFF cattail biomass.

4 CONCLUSIONS

The proof-of-concept of a non-land-dependent system based on GFFs for biomass production was successfully demonstrated in El Arenal (Spain), following the spirit of the LIFE programme, Actions for Environment and Climate. Green activities were implemented in a rural environment, counting with the involvement of farmers in the framework of the LIFE Biomass C+ Project. The chain of biomass production, from plant propagation to GFFs installment in farmers' irrigation ponds, and from biomass collection to characterization, was achieved.

GFFs of *Typha domingensis* performed well in view that water in irrigation ponds was oligotrophic, in general terms. Biomass production and C capture were consistent with water conditions. Biomass harvested at the end of the growth cycle, originated from a helophyte that was artificially grown as a floating plant presented properties within the range of values reported for rooted cattails. An unexpected finding from this work was the resilience of this plant species to these nutrient-depleted conditions.

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