NITROGEN AND PHOSPHORUS REMOVAL EFFICIENCY OF THREE HELOPHYTES IN CONSTRUCTED SURFACE FLOW WETLANDS FOR URBAN WASTEWATER TREATMENT

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ABSTRACT: Nutrient absorption is a function, among others, aimed at macrophytes for wastewater treatment. In this work, *Typha angustifolia*, *Phragmites australis,* and *Sparganium erectum* were multi-annually compared in secondary and tertiary treatment of urban wastewater under Mediterranean climate. *Phragmites* shows higher growth in height and density during the vegetative period of 1.83-2.09 cm.d^{-1} and 29-49 times the planting density, respectively. Aerial biomass records 52.2-54.3, 38.1-41.0, and 19.4 t dw.ha^{-1} in *Phragmites*, *Typha*, and *Sparganium* in the same order. The underground biomass fluctuates depending on the rooting vigour from 1.45 t dw.ha^{-1} for *Sparganium* to 44.49 t dw.ha^{-1} for *Phragmites* in tertiary treatment. Nitrogen and phosphorus aerial mineralomasses are more important in *Phragmites* (787 kg N.ha^{-1}) and *Typha* (107 kg P.ha^{-1}) in secondary treatment. *Phragmites* is, by far, the most cumulative of N and P in its underground part. The N and P retention by the vegetated mesocosms apparently has overall relationship with N-NH$_4^+$ and P-PO$_4^{3-}$. Compared to mesocosm input, *Phragmites* assimilates one's maximum of 6.39% N in its aerial tissues and 7.86% P in the underground ones, whereas *Typha* records maxima of 14.8% N and 33.6% P in its aerial part with respect to corresponding mesocosm removal.

ABSTRAK: Penyerapan nutrien adalah satu fungsi, antara lain, bertujuan untuk makrofit merawat sisa air. Kajian ini menggunakan *Typha angustifolia, Phragmites australis* dan *Sparganium erectum* bagi membandingkan pelbagai-jenis rawatan sekunder dan tertiar sisa air bandar pada iklim Mediterranean. *Phragmites* menunjukkan pertumbuhan tertinggi dalam ketinggian dan ketumpatan dalam tempoh vegetatif iaitu 1.83-2.09 cm.d^{-1} dan 29-49 kali ketumpatan penanaman, masing-masing. Rekod biojisim udara mencatatkan 52.2-54.3, 38.1-41.0, dan 19.4 t dw.ha^{-1} dalam *Phragmites, Typha*, dan *Sparganium* pada susunan sama. Biojisim bawah tanah berubah-ubah bergantung kepada kekuatan akar dari 1.45 t dw.ha^{-1} untuk *Sparganium* hingga 44.49 t dw.ha^{-1} untuk *Phragmites* dalam rawatan tertiar. Nitrogen dan Fosforus Mineralomassa udara adalah lebih penting untuk *Phragmites* (787 kg N.ha^{-1}) dan *Typha* (107 kg P.ha^{-1}) dalam rawatan sekunder. *Phragmites*, setakat ini, paling kumulatif bagi N dan P pada bahagian bawah tanah. Pembendungan N dan P oleh mesokisme tumbuh-tumbuhan secara nyata berkaitan dengan N-NH$_4^+$ dan P-PO$_4^{3-}$. Berbanding input mesokisme, *Phragmites* mengasimilasi maksimum pada N 6.39% tisu udara dan P 7.86% bahagian bawah tanah, manakala *Typha* mencatatkan maksima N 14.8% dan P 33.6% pada bahagian udara dengan penyingkiran mesokisme sepadan.

1. INTRODUCTION

The use of native macrophytes in constructed wetlands for wastewater treatment has often considered the biology of the species, particularly its life cycle, the tolerance of relatively high pollutant loads, a high productivity of valuable biomass, and local criteria such as climate, wastewater quality, and operating facilities of the treatment process [1-3].

Macrophytes obviously contribute directly or indirectly to the wastewater treatment process [3,4], and the direct absorption of nutrients is one of the desired functions in this regard. Harvesting aerial biomass of helophytes at the end of the growing season could enhance their productivity during the following season, and generally further increase the retention of nutrients, including nitrogen and phosphorus [5, 6].

The macrophytes most commonly used in the world in constructed surface flow wetlands (CSFW) correspond to species of *Typha, Scirpus, Schoenoplectus, Phragmites, Juncus* and *Eleocharis* genera [3], in addition to *Canna, Cyperus, Paspalum* and *Iris* for Mediterranean countries [7]. In Mediterranean Morocco, three helophytes were selected and tested in urban wastewater treatment within the framework of the "MHEA® experimental centre of M'Diq (NW of Morocco)" project, financed by the Walloon Region through the Agence de la Francophonie [8-10], *Typha angustifolia* L., *Phragmites australis* (Cav.) Trin. ex Steud. and *Sparganium erectum* L. These three species have been tested around the world to treat municipal, agricultural, industrial, and drainage waters [3].

Based on overall treatment performance, the experiments carried out in this respect focused on the development and optimization of new extensive wastewater treatment technologies, adapted to the regional climatic and socio-economic conditions (MHEA® processes), with three successive stages each composed of an artificial ecosystem or mesocosm [10].

This work specifically exposes for the first time a multi-year comparison of the three helophytes (*T. angustifolia, P. australis* and *S. erectum*) in secondary and tertiary treatments of urban wastewater, based on development descriptors with annual harvesting of biomass, and their direct contribution to the nitrogen and phosphorus removal in CSFW.

2. MATERIALS AND METHODS

2.1 Mesocosms

By imitating their respective habitats in natural or polluted environments, *T. angustifolia* (T), *P. australis* (Pr), and *S. erectum*(S), were grown separately, just before the vegetative period (VP), at a density of 10 rhizomatous fragments per m^2 in mesocosms by simulating macrophyte bioreactors: 2.2 m^3 of wet volume, including 1.8 m^3 of substrate and 0.4 m^3 of surface water, and an identical non-planted pond was designed for comparison. The substrate used consists of washed river sand with a particle size of about 38% (diameter <2 mm), 31.6% (2-5 mm), 30% (>5 mm) and 0.4% (<80 μm). The feeding of these mesocosms in pre-treated wastewater is done semi-continuously and in translation (CSFW). The three planted mesocosms are compared beforehand as secondary treatment, then in the presence of a control (non-planted mesocosm); the mesocosms with T or Pr are compared in secondary and tertiary treatments (Table 1).

The climate prevailing in the experimental site is of Mediterranean type: rainfall of 523 to 683 mm (1999-2002), average daily temperatures of 14°C to 26°C, maximum of the warmest months of about 35 °C (July and August), and minimum of the coldest months above 7°C (December and January). The strongest winds are recorded during the months of September (W-E), and November-December (S-N), with negative transient effects of salt spray and plant collapse. Water losses due to mesocosm evapotranspiration vary with planting type and treatment stage, from 5.28 to 19.0% of inlet flow (Table 2).

Table 1: Characteristics of inlets feeding mesocosms per treatment stage. Sources: [11-12]; unpublished data.

Mesocosms group	Secondary inlets (I2)		Tertiary inlet (I3)
	T. angustifolia(T2) *P. australis*(Pr2) *S. erectum*(S2)	Unvegetated control (L2) *T. angustifolia*(T2) *P. australis*(Pr2)	Unvegetated control (L3) *T. angustifolia*(T3) *P. australis*(Pr3)
DCO (mg $O_2.l^{-1}$)	402.9±236.2	292.2±148.9	34.81±14.63
DBO$_5$ (mg $O_2.l^{-1}$)	115.8±51.94	110.3±57.95	32.67±16.54
TN (mg N.l^{-1})	39.48±9.446	38.65±9.906	22.60±8.332
TP (mg P.l^{-1})	6.769±1.908	6.803±2.553	4.896±1.525
T (°C)	22.0±5.57	22.0±5.65	20.1±4.43
pH	8.24±0.58	8.07±0.61	7.48±0.19
O_2 (mg.l^{-1})	10.9±13.2	10.7±10.3	5.29±7.42
Conductivity (µS.cm^{-1})	1043.8±92.463	991.49±105.19	1136.9±120.23

On the other hand duckweed coming to settle in unplanted mesocosms, or in those planted after biomass harvesting, were regularly removed. *Diptera* and *Coleoptera* spontaneously succeed in the aquatic and aerial mesocosm compartments, with an affinity of *Cladocera* and *Gastropoda* for the first, and *Hemiptera* and *Hymenoptera* for the second. Furthermore, the Mosquito Fish [*Gambusia affinis* (Baird and Girard, 1853), *Poeciliidae*] was introduced throughout the VP into each of the three mesocosms in secondary treatment: T, Pr and into the un-vegetated one.

Table 2: Water balance in mesocosms per treatment stage. Sources: [11-12]; unpublished data.

	Mesocosm group	Inlet flow (l.d^{-1})	Evapotrans-piration (mm.d^{-1})	Retention time (d^{-1})
Secondary treatment	*T. angustifolia*(T2)	186.2±21.87	16.03±10.91	2.351±0.296
	P. australis(Pr2)	170.8±19.17	25.92±13.90	2.673±0.311
	S. erectum(S2)	210.2±23.60	23.55±13.39	2.152±0.272
	Unvegetated control (L2)	243.0±39.22	12.84±17.86	1.810±0.407
	T. angustifolia(T2)	217.7±42.16	16.80±19.79	2.096±0.592
	P. australis(Pr2)	224.9±50.46	16.03±17.53	2.072±0.715
Tertiary treatment	Unvegetated control (L3)	132.9±20.72	22.45±6.19	3.430±0.565
	T. angustifolia(T3)	137.1±21.37	16.57±11.36	3.254±0.642
	P. australis(Pr3)	144.4±22.50	27.47±10.91	3.244±0.629

2.2 Experimental Monitoring

Water samples are taken on each mesocosm inlet and outlet approximately every two weeks for analyses of nitrogen compounds (total nitrogen, ammonium, nitrates and nitrites) and phosphorus ones (total phosphorus and orthophosphates). In addition, water samples were also collected to quantify phytoplankton, and macrophyte development parameters were measured.

2.2.1 Nitrogen

Total nitrogen (TN) and total non-particulate nitrogen (TNf) are determined after digestion of the sample in an alkaline medium (NaOH) in the presence of persulfate whereby the nitrogen compounds convert to nitrates. Undigested persulfate is reduced by sodium metabisulfite. Nitrates react with chromotropic acid in a strong acid medium (H_2SO_4) to form a yellow complex with maximum absorbance at 410 nm. Two ranges are proposed by the Hach firm: the low range (0.2-25 mg N.l^{-1}) and the high range (7-150 mg N.l^{-1}).

Ammonium ions (NH_4^+) are determined according to the Nessler method on filtered samples. The hardness of the sample is complexed by a mineral stabilizer. The polyvinyl alcohol, a dispersing agent, helps to form a yellow colour with the Nessler reagent in the presence of the ammonium ions. The colouring developed is proportional to the concentration of ammonia. The reading is at 425 nm for a concentration range 0.05 to 2.5 mg N.l^{-1}.

Nitrite ions (NO_2^-) are determined according to the sulfanilamide method whose principle is the formation of a diazonium compound by the reaction of nitrites with sulfanilamide. This compound gives, by coupling with N-(1-naphthyl)-ethylenediamine dichloride, a red-mauve colour measured at 540 nm. The range used is 0.003 to 0.3 mg N.l^{-1}. Nitrate ions (NO_3^-) are reduced by cadmium to nitrite ones which react in acidic medium with sulfanilic acid to form an intermediate diazonium salt. The latter reacts with gentisic acid and forms an amber colour determined at 500 nm for the high range (0-30 mg N.l^{-1}) and at 400 nm for the medium range (0-5 mg N.l^{-1}). For the low range (0-0.5 mg N.l^{-1}), the diazonium reagent reacts with chromotropic acid and develops a yellow colour measured at 507 nm.

2.2.2 Phosphorus

Total phosphorus (TP) and total non-particulate phosphorus (TPf) are oxidized as orthophosphate ions in acidic medium in the presence of persulfate. Two ranges are used: (i) the low range (0-3.5 mg P.l^{-1}) for which orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdic complex which, in turn, is reduced by ascorbic acid giving an intense molybdenum blue coloration determined at 890 nm; (ii) the high range (0-100 mg P.l^{-1}) for which the phosphomolybdic complex forms in the presence of vanadium the yellow vanadomolybdophosphoric complex measured at 420 nm.

Orthophosphate ions (PO_4^{3-}) react, in acid medium, with the ammonium molybdate producing the molybdophosphoric acid. This complex is then reduced by the amino acid reagent to form the blue-molybdenum intensely coloured to determine at 530 nm. The method range is 0 to 9.8 mg P.l^{-1}.

2.2.3 Phytoplankton

According to the algae density in mesocosms, an adequate volume is taken at a depth of 10 cm. The phytoplankton biomass was evaluated by the chlorophyll-a determination in accordance with the spectrophotometric method specified in Standard Methods [13].

2.2.4 Helophytes

Macrophyte development parameters include stem density and height, biomass production, and N and P contents in their tissues. At the end of the VP the aerial part of each helophyte is cut above the water level, and its dead parts are collected separately to estimate respectively the above-ground biomass, and the necromass inside the mesocosm.

In addition during each VP, cuttings of the plant aerial part beyond mesocosms were sometimes required because of accessibility.

At the end of the 3rd VP, the mesocosms are emptied, and the rhizomes and roots of the middle 3rd are gently recovered and washed on stacked sieves with a regressive mesh for the estimation of the underground biomass. Representative subsampling of collected biomass is adopted, and the following analytical steps (drying, grinding, and N and P determination) were conducted according to Ennabili et al. [14].

2.3 Analytical Methods

Data are presented depending on three periods: vegetative period (VP), vegetative rest (VR) and "VP+VR". The one-way analysis of variance (ANOVA) of normal distribution data was performed using the STATISTICA software, V.5, otherwise only averages and standard deviations were calculated using Microsoft Excel software.

3. RESULTS AND DISCUSSION

3.1 Helophyte Development

3.1.1 Growth

Pr develops more in height especially in tertiary treatment (Pr3), successively followed by T and S (Table 3). These values greatly exceed those previously reported in T, Pr, and S grown in constructed wetlands, under polluted environments, or those developed in greenhouses, specifically 1.79-2.50 m for Pr, 0.90-1.99 m for T and 0.91 m for S [15-18], and are close to those underlined in natural or polluted environments, 3.2-3.5 m for Pr, 2.8 m for T and 1.70 m for S [6,14].

Table 3: Growth characteristics and above (A-) and underground (U-) biomass of helophytes studied per treatment level.

Mesocosms	T2	T3	Pr2	Pr3	S2
Height (m)	3.47±0.59	3.15±0.49	3.90±0.87	4.45±0.71	1.96±0.98
Stems.m^{-2}	86±52	38±24	494±217	289±140	79±31
Fertile stems (%)	17.58 ±14.00	43.59 ±67.48	20.43 ±10.61	17.49 ±8.044	34.78 ±26.65
A-dry weight (% wet weight)	19.47 ±4.969	19.60 ±4.623	39.33 ±1.343	43.13 ±3.721	15.17 ±6.536
A-Biomass (t dw.ha^{-1})	41.009 ±13.785	38.098 ±15.286	54.284 ±30.850	52.209 ±36.400	19.436 ±12.125
A-Necromass (t dw.ha^{-1})	5.850 ±5.459	6.220 ±7.269	7.310 ±9.914	2.745 ±3.627	9.075 ±10.60
A-partial pre-cutting (t dw.ha^{-1})	0.951 ±0.369	0.927 ±0.773	4.021 ±2.396	3.413 ±3.787	0.538 ±0.371
Net productivity (t dw.ha^{-1}.y^{-1})	59.895 ±4.2172	52.428 ±20.601	70.113 ±65.025	50.098 ±54.739	22.272 ±6.6706
U-Biomass (% ww)	6.80	18.47	22.20	22.27	6.78
U-Biomass (t dw.ha^{-1})	4.363	7.998	25.008	44.486	1.454
"U/A-Biomass" ratio	0.22	0.34	0.38	0.65	0.04

The average growth rate during the VP is 1.83-2.09, 1.48-1.63 and 0.92 cm.d^{-1} for P, T, and S in the same order, vs. 0.77-1.9 cm.d^{-1} underlined in Pr by Zheng et al. [6] and Zhang et al. [17]. The shoot collapse is spectacular in S especially during the "August-October" period as it was noted in a polluted environment by Ennabili and Gharnit [19], due to the cumulated effect of sludge discharge and wind. Asaeda et al. [20] point out that

collapsed shoots disappear in 40-60 days and constitute a large fraction of the floating organic matter.

Horizontal stems of Pr sometimes robustly branch out from rhizomes during fruiting (July), reaching 9 m in length with an average of 3 sprouts.m^{-1} (September), and becoming rhizome once into the inlet piped water of mesocosms. Aquatic stolons are also observed in T during the flowering-fruiting period.

Typically more apparent at the mesocosm margins, stem density at the end of VP increased by about 29-49, 3.8-8.6, an 7.9 times for Pr, T, and S, respectively, compared to the initial planting, exceeding for Pr the range of 130-175 plants.m^{-2} (17-18 times the planting density) highlighted by Zheng et al. [6], and the 107 plants.m^{-2} cited in a polluted environment [15]. The increased rates of "post-planting density" obtained by this author in greenhouse approach noted in this work, 53.0 and 8.47 times the planting density of Pr and S respectively, but substantially different from that of T (21.9 times). Those high values of densities are related, inter alia, to the high tillering rate in these species, particularly 15, 6 and 3 sprouts.plant^{-1}, respectively for Pr, T, and S [15], and to the annual cut of helophyte aerial parts [6].

Although peak flowering occurs in September, secondary flowering cohorts are noted along the VP: T (April, June-August), Pr (April, August), and S (April-May, July-August, October). The flowering rate is likely to vary inversely with the treatment stage, contrary to Pr (Table 3), and it is negatively affected in S by the shoot collapse just before flowering. However, this species completes more than one development cycle per VP, with a maximum of 3 during the 3rd year of mesocosm functioning. In addition, S seeds massively germinate from the mesocosm bottom or on water surface (Fig. 1). In addition, the sucking of S is also more remarkable in the canopy openings created by the shoot collapse in mid-summer.
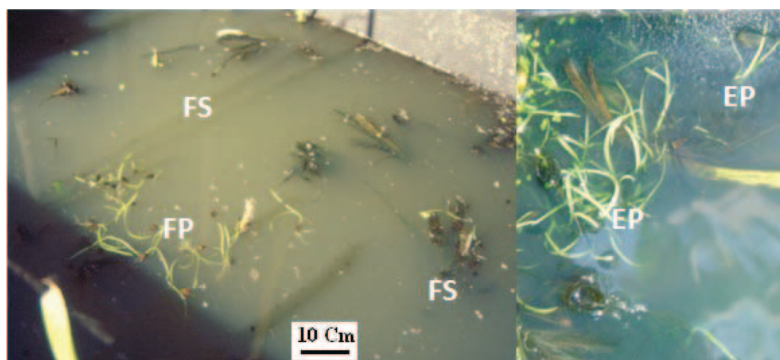

Fig. 1: Seed germination and development of *S. erectum*. FP, floating plantlets; FS, floating seeds; EP, emergent plantlets.

Moreover, the root bed is thicker in Pr, followed successively by T and S, and overwhelmingly achieves about 107, 56.7 and 20.6 cm deep from the level of substrate in the mesocosm middle part, respectively (Fig. 2). Liffen et al. [21] found that the biomass of S roots and rhizomes was highest at a depth of 0-10 cm, with a gradual decrease to 20-25 cm.

Rhizomes and roots of Pr go through the whole mesocosm substrate, forming root plaques at the bottom, and oxygenating the full height of the mesocosm bed except a slightly anoxic intermediate layer in appearance. The oxygen release from the Pr roots in the rhizosphere is qualitatively marked by a reddish colour, associated with the oxidized

forms of iron [4]. Pr rhizomes sometimes damage the mesocosm outlet by penetrating duct junctions.

3.1.2 Biomass

The above-ground biomass of helophytes is found to be slightly disadvantaged in tertiary treatment, with lower hydraulic loads (Table 2) and nutrients (Table 1), and ranges from 19.44 t dw.ha^{-1} for S2 to 52.21-54.28 t dw.ha^{-1} for Pr2, according to the lignification gradient, especially dry weights (dw) of 15.2 to 39.3-43.1% of the wet weight (ww) in the same order (Table 3). Which corresponds to a production in VP of about 0.193, 0.179, 0.255, 0.245 and 0.091 t dw.ha^{-1}.d^{-1} for T2, T3, Pr2, Pr3 and S2, vs. 0.14, 0.16 and 0.10 reported by Ennabili [15] in glasshouse, respectively for T, Pr and S.
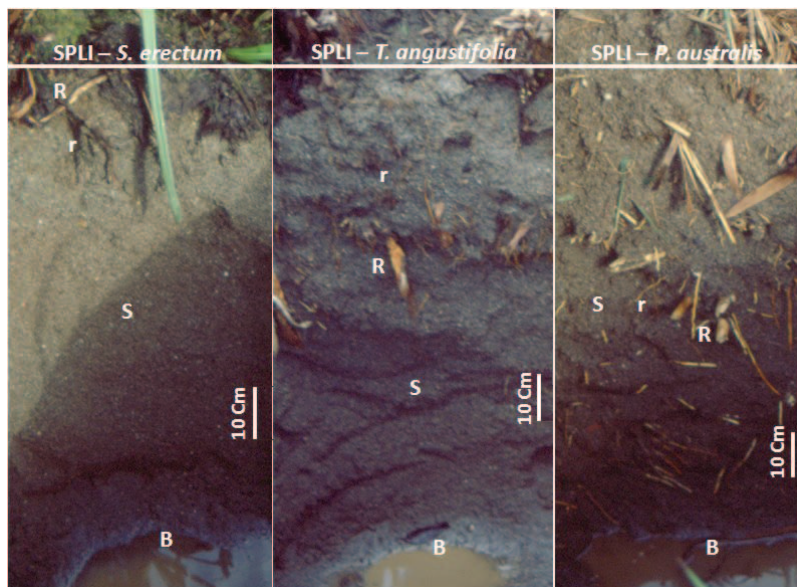

Fig. 2: Side view of dewatered mesocosm substrate after three vegetation periods. B, mesocosm bottom; R, rhizome; r, root; S, sand; SPLI, "sand/plant litter" interface.

Disregarding the role of study conditions, the aboveground biomass values obtained exceed those underlined in Pr (5.40-37.2 t dw.ha^{-1}, vs. 52.2-54.3 t dw.ha-1) [6,14-16,18, 22-25], were similar to those reported for T (7.76-33.0 t dw.ha^{-1}, vs. 38.1-41.0 t dw.ha^{-1}) [14-15,18,26], and were part of the range of values highlighted in S [14-15,20-21].

The above-ground biomass during three successive years with harvesting of helophyte aerial parts at the end of each VP shows that Pr has a relative stability of production in tertiary treatment, a resilience for Pr and S in secondary treatment, while the T biomass increases in the first two years and falls steeply in the third year compared to the 3-year average (Fig. 3). The positive impact of harvesting on biomass production has been demonstrated in Pr [6,25]. Due to non-harvesting of biomass de los Reyes et al. [26] showed that the T biomass improved by about 39 to 48% in the 3rd year for the above-ground and below-ground parts respectively, suggesting an inadequacy of medium and long-term harvest for these species growing in CSFW.

The partial cut of helophytes for accessibility along the VP exports from 0.075 t dw.ha^{-1} for S2 to 4.021 t dw.ha^{-1} for Pr2. The specific necromass collected after harvesting is higher for S (9.075 t dw.ha^{-1}), due especially to the shoot collapse, followed in descending order by those of Pr2, T3, T2 and Pr3 (Table 3), which would probably be a source of mesocosm enrichment during the forthcoming seasons. The net aboveground

productivity varies from more than 22 t dw.ha^{-1}.year^{-1} for S to over 70 t dw.ha^{-1}.y^{-1} for Pr in secondary treatment (Table 3), exceeding the estimated total biomass by Maucieri et al. [27] in Pr (49.2-58.9 ±9.1 t dw.ha^{-1}) in CSFW for agricultural wastewater treatment.

As far as the underground biomass, it fluctuates from 1.454 t dw.ha^{-1} for S to 44.486 t dw.ha^{-1} for Pr in tertiary treatment, approximately 4 to 65% of the respective aboveground biomasses (Table 3). These values overlap with those underlined for Pr (16.0-29.7 t dw.ha^{-1}, vs. 25.0-44.5 t dw.ha^{-1}) [14-15,23], but are outperformed by those reported for T (12.3-34.95 t dw.ha^{-1}, vs. 4.36-8.00 t dw.ha^{-1}) and S (6.49.48-23.5 t dw.ha^{-1}, vs. 19.4 t dw.ha-1) [14-15, 21, 26].
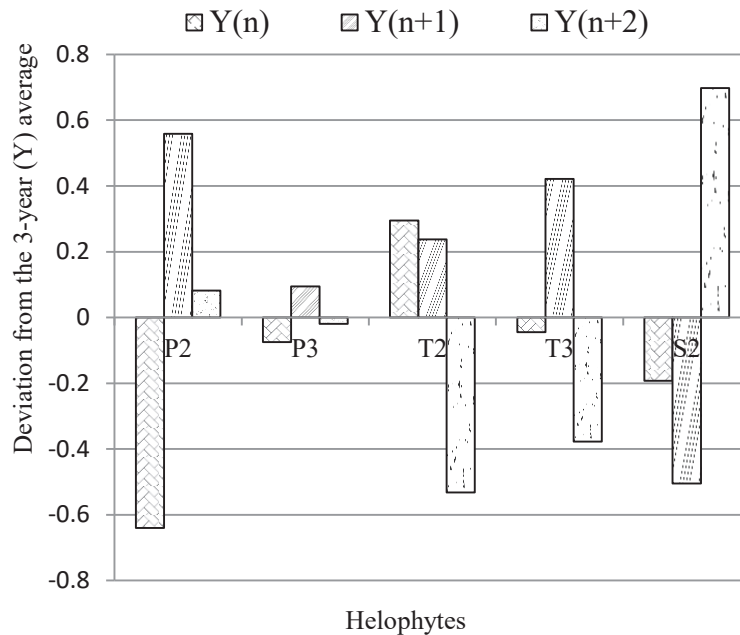

Fig. 3: Annual aboveground biomass in relation to the 3-year average one.

The above-ground biomass apparently is more favoured in CSFW (U/A of 0.04 to 0.65 depending on the species and the treatment stage) in comparison with the underground biomass (Table 3), contrary to the ratios obtained in polluted environments: 1.62, 1.62 and 0.55 for T, Pr and S in the same order [14]. However, by referring to the biomass averages obtained in these species from the same source by the same authors, and considering the harvest conditions at the end of each VP, CSFW systematically favour the aboveground biomass production (Fig. 4). The underground biomass is also favoured but with a lower intensity in Pr and T in tertiary treatment.

3.1.3 Mineralomass

The N/P contents in aerial tissues of helophytes are more apparent in Pr and T in secondary treatment, specially 787 kg N.ha^{-1}/89.6 kg P.ha^{-1}, and 670 kg N.ha^{-1}/107 kg P.ha^{-1} in the same order. The lowest rates for these two nutrients were recorded in T in tertiary treatment (Table 4). As regards the underground mineralomass Pr is the most cumulative of N and P, apparently due to its high underground biomass in CSFW.
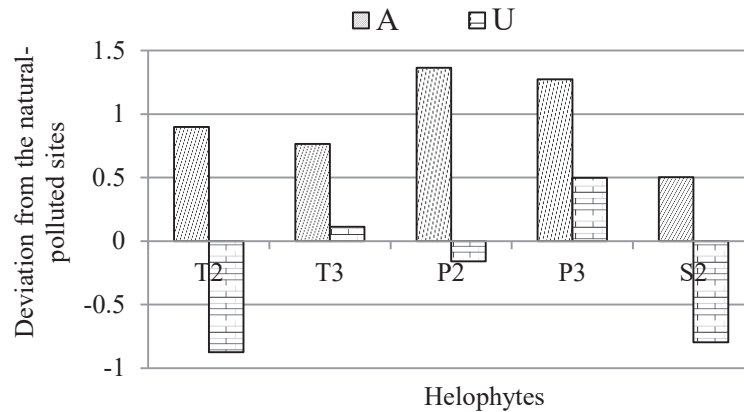
Fig. 4: Helophyte above-(A) and underground (U) biomass in relation to natural-
polluted sites [14] and CSFW (the present study).

Table 4: Nitrogen and phosphorus contents and mineralomass in above (A-) and
underground (U-) parts of helophytes studied per treatment level.

Mesocosms	T2	T3	Pr2	Pr3	S2
A-N (%)	1.64± 0.83	0.92± 0.25	1.45± 0.85	1.26± 0.47	1.90± 0.29
A-P (%)	0.26± 0.10	0.16± 0.08	0.17± 0.05	0.13± 0.01	0.45± 0.13
U-N (%)	1.23	0.62	1.00	1.08	1.08
U-P (%)	0.21	0.14	0.19	0.23	0.19
A-N (kg N.ha^{-1})	670.49±225.38	348.60±139.87	787.12±447.33	657.84±458.64	368.31±229.76
A-P (kg P.ha^{-1})	106.62±35.840	59.052±23.693	89.569±50.903	67.872±47.320	86.490±53.955
U-N (kg N.ha^{-1})	53.669	49.589	250.08	480.45	15.703
U-P (kg P.ha^{-1})	9.1629	11.197	47.515	102.32	2.7625

Compared to the dw, the N/P contents in helophyte tissues intersect those reported by other authors in Pr, except the low values of N in the underground part (Table 4), respectively 1.10-3.11/0.09-0.27% dw of the aerial part, and 1.44-1.73/0.22-0.32% dw of the underground one [2, 5-6,14,18]. For T and S the values underlined by other works are 1.94/0.09-0.17% and 1.94/0.35% dw of the aerial tissues, and 1.04/0.15% and 1.73/0.55% of the aerial tissues in the same order [14,18].

The mineralomass values reported by other studies clearly distinguish the N/P aboveground mineralomasses of Pr (658-787 kg N.ha^{-1}/68-90 kg P.ha^{-1}), vs. 111-502 kgN.ha^{-1}/17-37.4 Kg P.ha^{-1} [6, 14, 16, 24, 25, 27-29], and show an harmony of its N/P underground mineralomasses (250-480 kg N.ha^{-1}/48-102 kg P.ha^{-1}) vs. 503.3 kgN.ha^{-1}/32.02-76.9 kg P.ha^{-1} [6,14].

When compared with other authors, the N/P aboveground mineralomasses of T and S are similar (Table 4), vs. 74-420 kgN.ha^{-1}/20-55 kg P.ha^{-1} for T and 251-300 kgN.ha^{-1}/45.3-50 kg P.ha^{-1} for S [14, 26, 28, 29-31], while the N/P belowground ones are largely disadvantaged in this respect (Table 4), vs. 310-822 kgN.ha^{-1}/5-147 kg P.ha^{-1} for T and 124.3 kgN.ha^{-1}/39.5 kg P.ha^{-1} for S [14,26].

3.2 Phytoplanktonic Chlorophyll-a

The chlorophyll-a content in mesocosm water varies widely throughout the growing cycle. In secondary treatment and as compared to T and S (Fig. 5), the Pr-mesocosm generally reduces more phytoplankton (0.0171±0.0200 mg Chlorophyll-a.l^{-1}) and especially during the VR (0.0066±0.0069 mg.l^{-1}); this fact is more apparent in the T-

mesocosm in the VP (0.0174 ± 0.0196 mg.l^{-1}). In tertiary treatment, the Pr-mesocosm forcefully reduces phytoplankton (0.0038 ± 0.0105 mg.l^{-1}), compared to a lesser role of T-mesocosm, and an algae enrichment of the un-vegetated one (Fig. 5). This particular behaviour of the Pr-mesocosm is apparently in relation with, among other things, its leaf canopy more developed throughout the year.

In addition to the indirect role of helophyte shading on phytoplankton contraction, authors have shown allelopathic interferences of macrophytes with phytoplankton development. For example, extracts of leaves, stems and rhizomes of Pr can have negative effects on the growth of freshwater phytoplankton, *Microcystis* and *Scenedesmus* [32]. T and its allelochemicals can control phytoplankton blooms in eutrophic waters [33]. *Cyanobacteria* can also produce effective allelochemicals that interfere with the growth of competitive *Cyanobacteria* and algae [34].
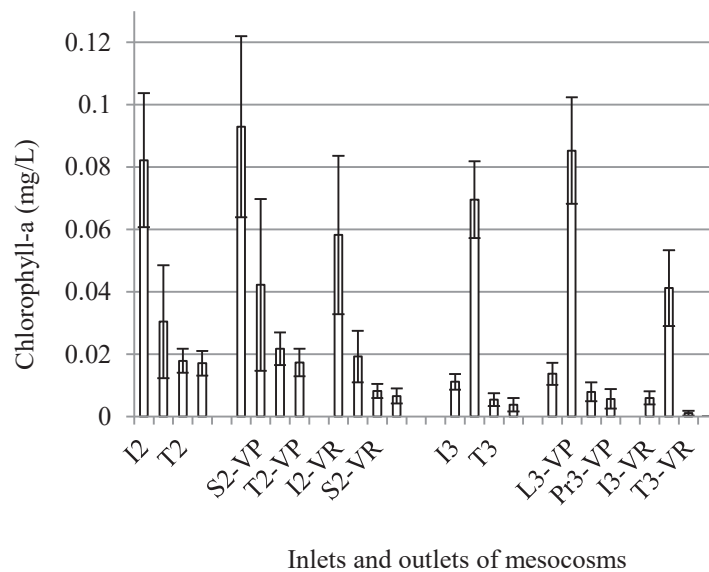

Fig. 5: Chlorophyll-a content of mesocosm inlet (I) and outlets (June 99-Sep.00).

3.3 Nitrogen and Phosphorus Removal Efficiency

In secondary treatment, the Pr-mesocosm has an advantage for the retention of TN, TNf and NH_4^+ in comparison with the T and S ones (Table 5), but it's similar to T-mesocosm and the non-planted one (L) for TN and TNf (Table 6). In tertiary treatment, it generates the best TN removal, and aggregates with L for TNf and NH_4^+, and with L and I (mesocosm inlet) for PO_4^{3-}(Table 7). S- and T-mesocosms would be of interest for the retention of NO_3^- and NO_2^-in secondary and tertiary treatments in the same order (Table 5 and 7).

The L-mesocosm shows significant removal of $NH_4^+$and TPf respectively in secondary and tertiary treatments, and generates similar concentrations of $NO_3^-$and $PO_4^{3-}$to those of I in secondary treatment (Tables 6 and 7). Depending on the I-water quality, planting of mesocosms apparently has no effect on the retention of $NO_3^-$and PO_4^{3-} in secondary treatment, and of PO_4^{3-}in tertiary treatment (Tables 5, 6 and 7). The impact of the VP on the N and P retention likely has a general relationship with the direct plant uptake of $N-NH_4^+$ and PO_4^{3-}, but remains without clear trend for TNf, NO_2^-, NO_3^- and TPf (Tables 5, 6 and 7), seemingly due to other factors such as bacterial activity, substrate composition, hydraulic retention time, etc.

Table 5: Nitrogen and phosphorus contents (mg.l^{-1}) of inlet and outlets of T-, Pr-,
and S-mesocosms (Secondary treatment; Jan. 99 – Sep. 00).

	Inlet	Outlets			ANOVA significance (p<0.05)		
	I2	T2	Pr2	S2	F-ratio	F-prob.	Effet
TN	39.5±9.45 a	31.6±8.33 b	22.8±10.36 c	31.2±8.96 b	21.37	0.0000	***
VP	38.3±9.39 a	31.6±8.07 b	22.9±10.74 c	30.1±8.56 b	13.62	0.0000	***
VR	42.5±9.35	31.5±9.37	22.6±9.77	34.0±9.79	-	-	-
TNf	26.6±8.09 a	25.7±6.91 a	19.0±7.67 b	25.5±10.36 a	6.597	0.0003	***
VP	26.0±8.83 a	26.3±7.16 a	19.5±7.85 b	25.7±10.54 a	3.852	0.0117	*
VR	28.1±6.02	24.1±6.28	17.9±7.48	24.8±10.4	-	-	-
NH$_4^+$	21.6±5.89 a	21.0±8.06 a	13.3±6.20 b	20.5±7.59 a	12.89	0.0000	***
VP	21.4±5.67 a	22.4±6.37 a	14.1±5.70 b	21.1±6.12 a	12.55	0.0000	***
VR	22.0±6.74	17.3±11.1	11.2±7.26	18.7±10.9	-	-	-
NO$_2^-$	0.016±0.021	0.014±0.020	0.019±0.018	0.008±0.008	-	-	-
VP	0.022±0.024	0.017±0.024	0.014±0.012	0.008±0.009	-	-	-
VR	0.005±0.005	0.008±0.007	0.030±0.023	0.007±0.006	-	-	-
NO$_3^-$	0.252±0.236 a	0.340±0.317 a	0.217±0.214 a	1.056±1.894 b	3.318	0.0243	*
VP	0.235±0.192	0.278±0.273	0.149±0.090	0.141±0.102	-	-	-
VR	0.278±0.303	0.432±0.372	0.320±0.301	2.428±2.477	-	-	-
TP	6.77±1.91 a	7.05±1.54 a	6.53±1.81 a	7.00±1.61 a	0.782	0.5058	NS
VP	7.00±1.72 a	7.31±1.30 a	7.02±1.73 a	7.20±1.68 a	0.242	0.8671	NS
VR	6.13±2.32	6.34±1.97	5.19±1.33	6.47±1.32	-	-	-
TPf	3.88±1.75	5.09±2.07	4.18±1.95	4.87±1.77	-	-	-
VP	4.27±1.56	5.64±1.64	4.60±1.88	5.11±1.81	-	-	-
VR	2.95±1.90	3.72±2.43	3.17±1.81	4.28±1.62	-	-	-
PO$_4^{3-}$	2.15±1.83 a	3.77±2.78 b	2.77±2.21 a	4.06±2.12 b	6.264	0.0004	***
VP	2.15±1.81	3.89±2.84	2.71±2.30	4.11±2.22	-	-	-
VR	2.18±1.99	3.45±2.71	2.93±2.03	3.92±1.90	-	-	-

For each variable, means followed by the same letter are not significantly different at 5%.
Non-significant (NS), significant (*), and very highly significant (***) effects.

The N/P retention in helophyte tissues compared to the I-mesocosm vary sharply and
reaches maxima of 6.39%/7.86% in Pr for the aboveground/underground parts (Fig. 6),
whereas by referring to the N/P removal rate by the corresponding mesocosms, T recorded
maxima of 14.8%/33.6% for the aboveground (Fig. 7), suggesting a greater retention of N
and P by the Pr-mesocosm compared to the T-one, contrary to the confirmation of Song et
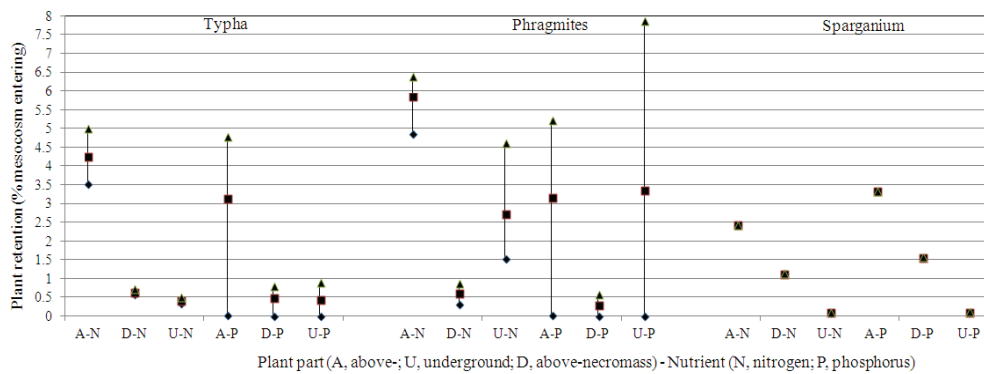al. [18] for P.

Fig. 6: Nitrogen and phosphorus retention in plant tissues in relation to the
corresponding mesocosm inlet.

Table 6: Nitrogen and phosphorus contents ($mg.l^{-1}$) of inlet and outlets of L-, T-, and Pr-mesocosms (Secondary treatment; May 01 – Sep. 03).

	Inlet	Outlets			ANOVA significance ($p<0.05$)		
	I2	L2	T2	Pr2	F-ratio	F-prob.	Effet
TN	38.7±9.91 a	32.6±9.46 b	31.4±9.17 b	28.5±9.35 b	11.18	0.0000	***
VP	41.0±10.2 a	34.7±10.3 b	33.7±9.44 b	29.8±10.1 b	7.289	0.0001	***
VR	34.9±8.38 a	29.1±6.77 b	27.7±7.52 b	26.4±7.81 b	5.053	0.0030	**
TNf	29.1±8.78 a	23.8±7.64 b	23.0±8.25 b	25.6±8.13 b	6.209	0.0005	***
VP	31.3±9.29 a	25.0±8.17 b	23.9±9.33 b	27.6±8.02 b	4.756	0.0035	**
VR	25.9±6.91 a	21.9±6.47 a	21.5±6.13 a	22.5±7.43 a	1.889	0.1377	NS
NH_4^+	23.8±7.57 a	18.2±6.73 b	21.3±9.00 a	22.8±8.39 a	5.756	0.0008	***
VP	26.0±7.97 a	19.4±7.28 b	23.2±10.4 a	25.6±8.55 a	4.881	0.0029	**
VR	19.8±4.77 a	16.0±5.10 a	17.8±4.23 a	17.8±5.35 a	2.156	0.0993	NS
NO_2^-	0.023±0.045	0.037±0.042	0.023±0.044	0.015±0.032	-	-	-
VP	0.023±0.042	0.030±0.035	0.022±0.042	0.010±0.022	-	-	-
VR	0.022±0.050	0.049±0.050	0.026±0.050	0.023±0.043	-	-	-
NO_3^-	2.80±1.82 a	2.62±2.16 a	3.91±2.03 b	3.85±1.73 b	7.301	0.0001	***
VP	3.28±2.00 a	3.31±2.31 a	4.43±2.29 b	4.39±1.80 b	3.530	0.0165	*
VR	1.98±1.10 a	1.43±1.13 a	3.00±1.00 b	2.91±1.10 b	10.81	0.0000	***
TP	6.80±2.55 a	6.57±2.76 a	5.96±2.37 a	6.03±2.07 a	1.731	0.1612	NS
VP	7.75±2.53 a	7.72±2.71 a	6.75±2.51 a	6.84±1.93 a	1.936	0.1261	NS
VR	5.12±1.55 a	4.54±1.31 a	4.55±1.18 a	4.58±1.42 a	0.910	0.4398	NS
TPf	4.20±1.87 a	3.84±2.09 a	4.59±2.23 a	4.47±1.83 a	1.653	0.1779	NS
VP	4.95±1.69 a	4.67±2.09 a	5.22±2.48 a	5.09±1.90 a	0.510	0.6756	NS
VR	2.88±1.39	2.36±1.01	3.46±1.02	3.36±1.04	-	-	-
PO_4^{3-}	2.94±2.01 a	2.71±2.16 a	4.02±2.15 b	3.95±1.84 b	6.751	0.0002	***
VP	3.47±2.22 a	3.40±2.31 a	4.59±2.42 b	4.52±1.94 b	3.300	0.0221	*
VR	2.00±1.08 a	1.48±1.11 a	3.03±0.99 b	2.94±1.08 b	10.86	0.0000	***

For each variable, means followed by the same letter are not significantly different at 5%.
Non-significant (NS), significant (*), highly significant (**), and very highly significant (***) effects.

For the whole plant, Pr assimilates in its tissues 10.9% N and 13.1% P compared to the I-mesocosm load. These values of N/P accumulated in aboveground plant tissues are far below than those obtained by de los Reyes et al. [26] in T (29.6% N) and Zheng et al. [6] in Pr (16.2-17.04% N). Besides, T is more effective in the N retention in its aerial tissues in secondary treatment and P in the whole plant in secondary treatment. Pr offers more efficiency of N absorption in its aerial part in secondary treatment and N and P in its underground part in tertiary treatment.

The underground part of Pr retains at most 5.23% N and 15.1% P of the general elimination rate of the corresponding mesocosm, and can be released progressively because of tissue death and decomposition during the forthcoming seasons. The S necromass in water is valued at up to 2.94% N and 7.96% P the general retention of the corresponding mesocosm, and will recover more rapidly in the treatment system given the relatively rapid decomposition of its collapsed shoots [31].

4. CONCLUSION

This work focused on the monitoring of *T. angustifolia, P. australis* and *S. erectum* grown in constructed surface flow wetlands for urban wastewater treatment in a Mediterranean climate, based on growth and functional criteria, and allowed an interspecific comparison under the same growing conditions on the one hand, and within each species by reference to natural-polluted environments on the other hand.

Table 7: Nitrogen and phosphorus contents (mg.l^{-1}) of inlet and outlets of L-, T-, and Pr-mesocosms (Tertiary treatment; Jan. 99 – Sep. 00).

	Inlet	Outlets			ANOVA significance (p<0.05)		
	I3	L3	T3	Pr3	F-ratio	F-prob.	Effet
TN	22.8±8.45 a	12.4±6.10 b	12.9±6.42 b	7.45±5.96 c	35.80	0.0000	***
VP	24.4±9.38 a	14.2±6.65 b	15.4±6.34 b	7.68±4.92 c	24.23	0.0000	***
VR	20.1±5.97	9.53±3.66	8.73±3.97	7.07±7.56	-	-	-
TNf	17.7±5.29 a	7.26±3.92 b	10.4±6.02 c	5.84±4.41 b	43.00	0.0000	***
VP	18.4±5.39 a	6.88±3.57 b	12.0±6.23 c	5.56±3.68 b	36.29	0.0000	***
VR	16.3±5.01	8.00±4.56	7.38±4.37	6.38±5.69	-	-	-
NH$_4^+$	14.0±4.81 a	3.17±2.23 b	7.30±4.51 c	2.74±2.54 b	82.06	.00000	***
VP	15.2±4.27 a	3.42±2.57 b	8.53±4.81 c	2.99±2.63 b	68.87	0.0000	***
VR	11.18±4.92	2.61±1.05	4.73±2.45	2.38±2.35	-	-	-
NO$_2^-$	0.065±0.053	0.121±0.090	0.021±0.034	0.024±0.028	-	-	-
VP	0.073±0.064	0.116±0.099	0.020±0.040	0.018±0.025	-	-	-
VR	0.051±0.018	0.132±0.073	0.024±0.022	0.035±0.030	-	-	-
NO$_3^-$	0.779±0.598 a	0.743±0.951 a	0.448±0.731 a	0.245±0.344 a	2.694	0.0519	NS
VP	0.679±0.653	0.428±0.773	0.122±0.047	0.132±0.114	-	-	-
VR	0.929±0.507	1.214±1.044	0.936±0.996	0.413±0.497	-	-	-
TP	4.94±1.52	4.24±1.81	4.46±1.56	3.82±1.81	-	-	-
VP	5.49±1.48	4.75±2.08	5.13±1.49	4.41±1.99	-	-	-
VR	4.14±1.17	3.48±0.81	3.50±1.04	3.01±1.06	-	-	-
TPf	3.05±1.42	2.31±1.09	3.15±1.30	2.66±1.71	-	-	-
VP	3.40±1.30	2.38±1.09	3.56±1.24	3.11±1.85	-	-	-
VR	2.46±1.47	2.18±1.13	2.47±1.15	1.90±1.10	-	-	-
PO$_4^{3-}$	2.32±1.39 a	2.17±0.93 a	3.07±1.30 b	2.26±0.84 a	5.360	0.0015	**
VP	2.41±1.33	2.29±0.97	3.43±1.31	2.49±0.78	-	-	-
VR	2.14±1.56	1.91±0.80	2.29±0.92	1.77±0.79	-	-	-

For each variable, means followed by the same letter are not significantly different at 5%.
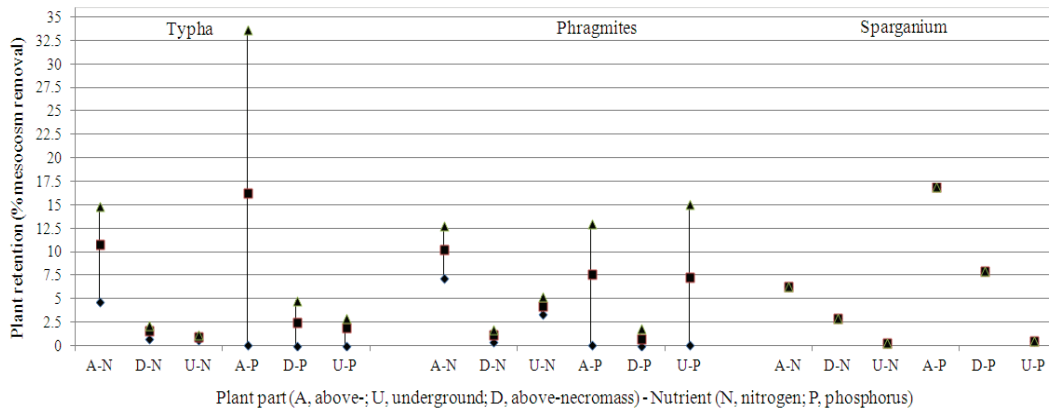Non-significant (NS), highly significant (**), and very highly significant (***) effects.

Fig. 7: Nitrogen and phosphorus retention in plant tissues in relation to the corresponding mesocosm efficiency.

In Mediterranean climate the *Phragmites* mesocosm is more effective in plankton reduction especially in tertiary treatment, compared to a lesser role of the *Typha* and *Sparganium* ones. By helophyte harvesting at the end of each vegetative period, there is relative stability of biomass production by *Phragmites* in tertiary treatment, resilience for *Phragmites* and *Sparganium* in secondary treatment, while *Typha* increases biomass

production the first two years and falls steeply the third year. Moreover, the aerial biomass of the three helophytes seems to be slightly disadvantaged in tertiary treatment.

Given the development magnitude of its underground biomass, *Phragmites* is by far the most cumulative of nitrogen and phosphorus in this part, in addition to the indirect benefit of substrate oxygenation, an interesting aspect for wastewater treatment. *Phragmites* also accumulates more nitrogen in its aboveground part in secondary treatment, and the nitrogen and phosphorus in its underground part in tertiary treatment. In secondary treatment *Typha* shows a specific interest in the assimilation of nitrogen in aerial tissues, and phosphorus in the whole plant.

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