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Re-naturalization of a drainage-based Mediterranean peatland: floristic and vegetation traits, biomass production and nutrients uptake

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Abstract

A pilot experimental field combining peatland rewetting and water phyto-treatment was set up in the Massaciuccoli Lake basin (Tuscany, Italy) to reduce the water eutrophication and peat degradation caused by almost a century of drained-based agricultural use.

In this paper, we investigated the re-naturalization process occurring consequently to the conversion of a peatland area in a natural wetland system (NWS) (the partial top soil removal, the realization of a perimeter levee to contain the waters, the rewetting with the waters coming from the drainage of surrounding areas) and the capability of the spontaneous vegetation to catch nutrients acting as a vegetation filter.

To follow the re-naturalization process over time (2012-2016), we used a mixed approach merging phytosociological survey with ortophotos taken by a drone. During the last year of observation (2016), we performed destructive sampling on the vegetation groups most represented in the area (*Phragmitetum* and *Myriophylletum*) to quantify of the biomass production and the nutrients (nitrogen and phosphorus) uptaken.

Phragmites.australis (Cav.) Trin. ex Steud. yielded significantly more than *Myriophillum aquaticum* (Vell.) Verdc. (4.94 kg m^{-2} *vs* 1.05 kg m^{-2}). Although *M. aquaticum* showed higher nutrient contents (2.04% N and 0.35% P), *P. australis* was able to uptake more nutrients within the NWS because of its larger cover and productivity.

In a management perspective of a natural wetland system for phyto-treatment and re-naturalization purposes, the authors suggest 4-5 year-long-harvesting turns, better occurring in spring-summer, to maximize the quantity of biomass harvested and the amount of nutrients temporarily immobilized in plant vegetative tissues.

Keywords:

Phragmites, Myriophyllum**,** phyto-treatment**,** drone, phytosociology

Highlights:

Rewetting agricultural drained peatlands can contribute to increase the diversity of species in these ecosystems

Using a mixed approach based on remote sensing and phytosociological surveys can facilitate the monitoring on large scale

In a rewetted peatland, spontaneous vegetation, if properly managed (harvested), can contribute to reduce eutrophication of water and slow down subsidence

1. Introduction

Peatlands drainage and their following exploitation have severely compromised their ecological and biological status worldwide, due to the changes in the land use by agriculture, forestry and urbanization (Grootjans et al., 2012). We can estimate that nowadays less than 20% of the original, pristine wetland areas still remain (Verhoeven et al., 2014).

By altering groundwater patterns and compositions, extensive peatland drainage has determined significant changes on the physics and chemistry of peats, leading to: i) acceleration of organic-matter oxidation (Oleszczuk et al., 2008), with a consequent increase in greenhouse gases (GHG) emissions into the atmosphere of up to 25 t CO_2 equivalent $ha^{-1}y^{-1}$ (Wichtmann and Wichmann, 2011; Couwenberg et al., 2011); (ii) enhancement of mineralization and nitrification of organic N due to higher oxygen availability and consequent increase of NO_3^- concentrations in porewater (Tiemeyer et al., 2007) and (iii) mineralization of organic P compounds and increase of absorbed and Fe-bound P pools (Zak et al., 2004). The continual recurrence of these phenomena has negatively affected the status of peatlands, lowering the soil level (subsidence), increasing nutrient loads delivered to receiving water bodies (eutrophication) and decreasing ecosystem biodiversity and functionality (loss of resilience) (Smolders et al., 2006; Pistocchi et al., 2012; Lamers et al., 2015).

Moreover, these deeply drained areas are becoming also unsuitable for modern agricultural production requirements (Pfadenhauer and Grootjans, 1999) and almost inaccessible for the ordinary machines used in agriculture.

From a merely biodiversity perspective, peatlands are unique, complex ecosystems of global importance for species and ecosystem conservation, since they contain many species found only or mainly in peatlands and their presence is strictly linked to the water regime of these areas (Tanneberger and Wichtmann, 2011).

All these above-mentioned factors considered, stopping the peatland drainage and planning the consecutive management represent an environmental priority to face.

From the different restoration case studies reported by literature, we can derive that there are different reasons leading to the change moving from the traditional drainage-based management of peatlands: stimulating the re-naturalization of portion of the land deteriorated from prolonged drainage (e.g. restoring) or recovery of the agricultural productivity of the areas (e.g. paludiculture).

Regardless the scopes behind the restoration, we can assume that to achieve the rehabilitation of at least some of the functions supplied by these ecosystems, the two most important conditions that cannot be missed are i) rewetting (e.g. constructing dams or filling in drainage ditches) and ii) reduction of trophic status (e.g. by mean of the top soil removal) (Van Dijk et al., 2007; Klimkowska et al., 2010 a,b; Zak et al., 2014).

Raising water level and flooding organic soils may contribute to lower the soil nutrient availability (mainly released as ammonium), but at the same time can boost phosphorus mobilization (Lamers et al., 2002; Meissner et al., 2008; Zak et al., 2004).

Top soil removal is the removal of the upper most degraded peat layers and it has been evaluated responsible of the higher mobilization of phosphorous during the rewetting of peatlands according to results of both lab and field researches (Zak and Gelbrecht, 2007; Zak et al., in press). Moreover, it can cause the removal of the reproductive organs of plant species (seeds, stolons, rhizomes, etc.) from the wetland habitats (Leps, 1999).

The case we report in this paper is about a project realized in Tuscany (IT), which compares three different management strategies aimed to combine the peatland rewetting and the water phyto-treating action. The three strategies differ among them for the level of anthropogenic presence (construction works, soil handling, plant species plantation, etc.) that they have required: constructed wetland system (CWS), paludicultural system (PCS) and natural wetland system (NWS).

In this paper, we focused on the NWS. The first objective was to follow the re-naturalization process after rewetting of lowlands to test whether it can re-establish the typical mire community in a relative short time. According to literature, the ecological restoration perspective is highly dependent on the zero-point condition before starting the restoration process. Indeed, while Tanneberger and Wichtmann (2011) report that top soil removal in combination with rewetting can lead to the restoration of soft-water pools and small sedge marshes within 5 years, Poschlod (1992) shows that in the case of peatland severely used for peat extraction mostly monospecific stands of non-peat-forming species could develop even for 20 years after the rewetting. Joosten (1995) even reports that in the cases of severe anthropogenic impact on the environment, it is not possible to observe any change within a human time perspective.

A second important research goal was to evaluate the capability of the NWS to abate the nutrients loads from waters to treat coming from the nearby rural district through the quantification of the biomass production and nutrient uptakes of plants spontaneously developed in this area.

2. Material and Methods

2.1 Site description

The study was carried out over 5 years (2011-2016) in Vecchiano, about 10 km from Pisa, Italy (43° 49’ 59.5’’N; 10° 19’ 50.7’’) in the Migliarino, San Rossore, Massaciuccoli Natural Park, within a 15 ha experimental area (Fig.1).

This area was used to compare the efficiency of three different strategies (Fig.2) in treating the eutrophic drainage water coming from a sub-watershed within the reclamation district around the Massaciuccoli lake. In this area, phosphorous has been recognized as the primary limiting factor for the eutrophication and the losses of this nutrient from cultivated fields (dissolved + particulate fractions) are estimated in 2-4 kg $ha^{-1}y^{-1}$ (Pensabene et al., 1997; Bonari et al., 2013).

The NWS was set up as natural rewetted area with a surface area of 2.7 ha and surrounded by small enbankments built with the top soil removal (~ 10 cm) carried out long the area's borders. Natural elevation changes within the NWS helped in creating zones with a different water level in order to promote the colonization from large plant species.

The soils of this experimental area can be classified as Histosol according to the USDA system and as Rheic Histosol according to the FAO system (Pellegrino et al., 2015).

The climate is classified as Mediterranean (Csa) according to Köppen-Geiger climate classification map (Kottek et al., 2006). Summers are dry and hot, while rainfall is mainly concentrated in autumn and spring (mean annual rainfall = 910 mm) and mean air temperature at 2 m ranges from 6.6 °C to 21.8 °C (mean= 14.6).

Mean monthly temperatures and rainfall for 2011-2016 were recorded at a weather station closed to the experimental site, located in Metato (San Giuliano T., 5 m s.l.m., 611370 E UTM, 4847363 N UTM)(Fig.3).

The water level within the NWS was registered daily from December 2013 to August 2016. From January 2013 to November 2013, the system was not equipped with a diver for the water level measurement, thus manual measurements were performed every 15 days (Tab.1).

The analyses of treated waters confirmed their eutrophic status with average annual total nitrogen content ranging from 8.13 mg/L to 7.14 mg/L, the average annual total phosphorus content ranging from 0.24 to 1.07 mg/L. About the soluble forms, the average annual Soluble Reactive Phosphorus (SRP) ranged between 0.15 and 0.22 mg/L, while the average annual nitrates content varied from 1.41 to 3.23 mg/L.

2.2 Trial setup and vegetation analyses

The construction works were prolonged between 2011 and 2012, while in January 2013 the phyto-treatment system started to operate. After a first recognition on the flora carried out in spring 2010 (before the beginning of the construction works), the NWS was periodically monitored from April 2013 up to July 2016.

The nomenclature of the plants accords to Pignatti (1982) and Conti et al. (2005). Every year, in spring-summer, vegetation development and phytocenotic diversity were surveyed using the Braun-Blanquet method (Braun Blanquet, 1979). During the monitoring period of NWS, aerial photos of the NWS were taken, every summer, from a drone (Iris +, 3D Robotics) flying at 15 m of height and equipped with a camera (gopro hero 3 +, 12 Megapixel). After georeferencing and straightening each photo, the high resolution (1-2 pixel) aerial photos were imported in GIS environment using Map Info ® 10.1 for the composition of the orthophoto mosaic. The images were interpreted assigning each digitized area to a specific phytosociological community.

In July 2016, we performed a destructive sampling aimed at the determination of the biomass production and nutrient concentration of the plant species grown within the NWS. The sampling plots were chosen on the basis of the vegetation map assembled after the flight of June 2016. Within each mono-specific populations with coverage of nearly 100%, we identified representative plots for the sample collection. In relation to their covered surface, for the two most represented plant species, we identified 7 homogenous sampling areas for *Phragmites australis* (Cav.) Trin. ex Steud. and 3 for *Myriophyllum aquaticum* (Vell) Verd.(Fig. 4). For each of these areas, we took 3 samples using a metal frame with an inner area of 1 m^2. Within the square surface, the biomass of *P.australis* was harvested above the water surface level, while in the case of *M.aquaticum* we took both the aerial and submerged parts (emergent shoots, stolons and submersed shoots), that is almost the whole plant biomass (Sytsma and Anderson, 1993), All sampling was done from a boat to minimize disturbance of the ecosystem.

For the determination of dry mass, samples were dried at 60 °C until reaching constant weight. The calculation of the total biomass produced by NWS during 2016 was obtained by multiplying the average of biomass samples collected within the plots belonging to the same homogenous population for their surface and then summed one to the others.

To determine the composition of biomass, samples were finely milled with a Fritsch Pulverisette 14.

Nitrogen and Carbon concentration was measured with a CHNS Analyzer (Vario EL III - Elementar Analysessysteme Hanau Germany) through gas chromatography after dry combustion at 1150 °C (DIN EN 15104, 2010; DIN EN 14961-1, 2010). Phosphorus concentration was determined through spectrophotometric determination after acidic digestion in a microwave digestion.

To validate the laboratory methods, a standard biomass of *P. australis* (Netherlands, BIMEP 412) was used (WEPAL, 2011).

The quantity of nutrient uptaken by plants was calculated as the product of the dry matter biomass production per surface unit and the nutrient concentration.

Data were analyzed with ANOVA according to a complete randomized block design (CRD), with the homogeneous area for sampling as factor replicated three times (with each elementary sampling area of about 1 m^2). The Duncan honest significant difference test was used for post hoc means comparison at the 0.05 p-level. The N, P and C concentration data were transformed in arcsine to fulfil the assumptions of ANOVA (version 9.1; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Flora and vegetation

The floristic census conducted before the beginning of construction works (October 2011), showed the presence of 17 botanical species that were partly synanthropic species (12) and partly wild species (5), typical of the nearby palustrine areas such as *Phragmites australis*, *Typha Latifolia* and *Lithrum salicaria* (Tab. 2).

During the last survey (May 2016), 21 plant species were identified within the NWS' embankments. All these species were hydro-hygrophile typical of wetlands and strictly related to palustrine and marshy ecosystems in Italy (Pignatti, 1982)(Tab.3).

The phytosociological surveys led to the identification of one association (*Phragmitetum australis* (Gams 1927) Schmale 1939) and three plant communities not exactly referable to any known syntaxonomical formation (Tab. 4,5,6,7) (Bertacchi et al., 2015).

In the case of *Phragmitetum australis*, data analyses confirmed the equipollence between the structure of the surveyed association and those of associations widely spread on the typical lacustrine areas of Tuscany as well as described by Tomei et al., (1998).

In the other cases, only homogeneous vegetation groupments were identified. The exiguity of the phytocenosis (*Typha latifolia*), the monophytic character of the vegetation (*Myriophyllum aquaticum*) or the serial stage still in progress (wet meadow, which has been surveyed only from June 2014), did not allow us to reach a reliable assignment.

3.2 Photointerpretation and vegetation mapping

The ortophotos taken from the drone and re-organized one next to the other to recompose the whole area, allowed us to draw the development dynamics of the four above-cited plant communities over time (Fig.5).

The implementation of the reconstructed photographic mosaic in GIS and the processing of digital data provided useful information about the fluctuations of the spatial pattern for the identified plant communities (Fig. 6).

In Figure 5 we reported the trends from 2012 to 2016. The area covered by *T. latifolia* was the lowest and almost stable in time (average 0.03 ha) while the area covered by wet meadow was, on average, the largest even if with wide fluctuations among the years (2.30 ha in 2013, 0.60 ha in 2014, 0.02 ha in 2015 and 0.16 ha in 2016). The *M. aquaticum* pointed out a significant increase of the covered surface between 2013 and 2015 (from 0.04 ha to 0.70 ha) whereas it has been more than halved in the last year (0.34 ha). The development of the *P. australis* showed shorter variations over time, increasing from 0.60 to 0.96 ha in the 2013-2015 period and drawing a little contraction in 2016 (0.78 ha).

Overall, the total vegetation cover of the area decreased from 2.24 ha in 2013, to 1.82 ha in 2014, to 1.68 ha in 2015 up to 1.30 ha in 2016.

3.3 Biomass production and nutrients uptakes

The ANOVA analyses results are reported in Table 8.

In general, *P. australis* proved to be significantly more productive than *M. aquaticum*. *P. australis* growth was almost homogeneous within the NWS' embankments with the only exception of the sub-area n. 9, where the biomass production was significantly higher than those recorded in the other sub-areas.

The mean production for *P. australis* was 4.94 kg m^{-2} d. m. while for *M. aquaticum* was 1.08 kg m^{-2} d. m. The values of biomass per unit area ranged from 3.15 kg m^{-2} d. m. (sub-area n. 4) to 6.44 kg m^{-2} d. m. (sub-area n.9) for *P. australis* and from 0.8 kg m^{-2} d. m. (sub-area n. 6) to 1.44 kg m^{-2} d. m. (sub-area n. 7) for *M. aquaticum.*

Summing the biomass production of each homogenous area, we obtained a total biomass production of 36.52 tons d. m. for *P. australis* and of 2.90 tons d.m. for *M. aquaticum* deriving from an occupied surface equal to 0.74 ha and 0.23 ha respectively.

About macronutrient concentration in biomass, there was not a significant difference in the nitrogen content between *P. australis* and *M. aquaticum*, neither among the different sampling areas. The values detected ranged between 1.55% and 2.20%.

Conversely, phosphorus concentration changed significantly depending on both the plant species and the sub-areas. The higher content was registered for *M. aquaticum* whose sub-areas showed always largest values of the NWS' embankments (0.44% for the sub-area n. 6 and 0.30% for the sub-areas n. 7 and 10). The lowest values were registered for *P. australis* that showed contents ranging between 0.14% and 0.19%.

The carbon content also varied significantly related to the species and sub-areas considered. The highest values was detected for *P. australis* from the sub-area n. 4 (45.58%) which was statistically different from the samples of the same species collected in sub-areas n. 1, 2, 5 and 9 (from 44.15% to 45. 36%) and in the sub-area n. 3 (44.05%). The carbon concentrations for *M. aquaticum* resulted even lower, equal to 44.52% in the area n. 10 and about 39% in the sub-areas n. 6 and 7.

The average nitrogen concentration detected in *P.australis* was 1.79, while in *M.aquaticum* was 2.04%, while about phosphorus, the average concentration in *P.australis* was 0.17% and in *M.aquaticum* was 0.35%.

We can derive that the total nitrogen taken up from the spontaneous vegetation was 707 kg, of which 657 kg taken up from *P. australis* and the remaining 50 kg taken up from *M. aquaticum*.

About phosphorus, *P. australis* contributed to take up 62 kg while *M.aquaticum* to 9 kg.

From the all results gained about productivity and nutrient uptakes, a part of a species effect, we could not identify an effect related to a different nutrient availability mainly due a particular drainage water flow path within the NWS.

4. Discussion

4.1 Geobotanical considerations – Vegetation response to rewetting

From six years of observation, we can derive that the water level rising was successful determining an increase of the absolute number of hydro-hygrophilous species (Tab.2). Moreover, the species found were similar to with those of the lacustrine ecosystems nearby in the Lake, especially for the inner areas of NWS.

A critical phenomenon can be represented by the presence and by the significant development of *M. aquaticum.* This exotic species of South American origin, in Italy is considered invasive and can pose a threat to other hydrophitic indigenous communities (Lastrucci et al., .2005). In this case, however, the species was already present in the area of the lake Massaciuccoli and was subsequently penetrated the NWS system.

About the vegetation, it was evident the predominance of the hygrophylous phytocoenosis, which showed a quick renaturation process, as evidenced by the wide surface covered by the different plant communities.

The spatial distribution of the vegetation species within the area showed the prevalence of the *Typha* on the borders or in the strips surrounding the central nucleus of NWS system colonized by the *Phragmites* population because of its higher competitive attitude. Indeed, as reported by many authors, *Phragmites* shows a great competitiveness under eutrophic conditions (Findlay et al., 2002; Meyerson et al., 2002)

The presence of a permanent water layer (30-35 cm) upon the soil could justify the large distribution of *Phragmites* and *Myriophyllum* within the NWS system. These species can prevail for long time and limit the development of other plants, such as *Carex spp.* or *Juncus spp.* This behavior seemed to be proved by the fact that the serial stage of wet meadows phytocenosis was surveyed only during a temporarily drying up of some portions of the NWS (mostly occurred nearby the borders). This means that only the regression of *Phragmites* and *Myriophyllum* communities due to the drought conditions allowed wet meadows to evolve toward next serial stages of phytocenosis.

The stabilization of the *Phragmitetum* extension, occurred in 2016, was matched with that of the *Myriophylletum* and can be related to their different ecological requirements, specific competitiveness and environmental conditions. Indeed *P. australis* is a rhizomatous helophyte, that needs to keep the overwintering buds under water but for a part of the year prefers to be in almost dry condition. The permanent flooded conditions in our cases may have favored the hydrophytes as *M. aquaticum* which spread in almost all the free spaces available.

All considered, we can speculate in term of phytocenosis development perspective, a small fluctuation of their specific cover without any significant overlaps between them. The climax stadium has almost been reached, and if ‘adverse events’ (e.g. harvest, fire) would not occur, *P.australis* would gradually occupy all the available spaces.

4.2 Photointerpretation and vegetation mapping

The mixed investigation method proposed, which merges field surveys and remote sensing data, can be particularly useful for the identification of the vegetation dynamics fallowing a renaturation process. It seems to be promising since it permits the integration of different scale methods by combining the accuracy of a field level survey (repeatability and the detectability) with the potentiality offered by remote sensing to extend the monitoring over huge areas (Stroh and Hughes, 2010). Moreover, the proposed method is rather simple to use and it does not require the purchase of expensive devices or sophisticated knowledge of computer science or drones management.

4.3 Biomass production and nutrients uptakes

The high productivity of *Phragmites australis* could be explained by a series of favorable conditions such as abundant water, high incident radiation, favorable growth temperatures, highly nutrient availability and the sampling time choice (coincident with the highest biomass productivity) as it is also confirmed by several studies conducted in nutrient-rich environments.

For example, Hocking (1989) registered 9.89 kg m^{-2} d. m. at the peak of productivity in a nutrient-enriched swamp in inland Australia, Gopal and Sharma (1982) reported production ranging from 7.50 to 9.30 kg m^{-2} d. m. in nutrient enriched wetlands in India, while more recently Eid et al., (2010) showed a production of about 5.40 kg m^{-2} d. m. in Egypt (Burullus Lake), which is quite similar to our average production (4.94 kg m^{-2} d. m.).

In conditions of nutrient limited availability, Aseada et al. (2002) reported a much lower productivity ranging from 9.81 to 1.03 kg m^{-2} d. m. (Austria), Karunaratne et al. (2003) reported 1.98 kg m^{-2} d. m. (Japan) while Aseada et al. (2006) reported 0.69 kg m^{-2} d. m. (Japan).

About *Myriophyllum aquaticum*, also mentioned in literature as parrot feather, we could expect a remarkable biomass production, since from many studies it is reported a positive correlation between relative growth rate and nutrient availability (Hussner et al., 2008). The average yield we registered (1.08 kg m^{-2} d. m.) was similar to those reported by Sytsma and Anderson (2003) for a Californian experience (1 kg m^{-2} d. m.), but still lower if compared with data reported by Shymayana (1988) (2.80 kg m^{-2} d. m ., assuming a fresh weight/dry weight ratio equal to 0.21) or even more with those reported by Monteiro and Moreira (1990) (about 4.60 kg m^{-2} d. m.).

In consideration of the phyto-treatment purpose attributed to the re-naturalization process, the capability of the plants of taking up nutrients from waters and storing them in their tissues can play an important role in determining the efficacy of this option. This feature is extremely important for phosphorus, for which the plant removal and subsequent harvesting represents the main means to cut down the nutrient loads in the water basin; differently, for nitrogen the role played by plants in its removal is lower considered that it can be lost also through a gaseous phase (Vymazal, 2007).

In our study, the mean amount of nutrients removed were high for *P. australis* (~ 894 kg N ha^{-1} and ~85 kg P ha^{-1}) and although lower, as well remarkable for *M. aquaticum* (~211 kg N ha^{-1} and ~36 kg P ha^{-1}).

These quantities can be considered not so high if compared with the nutrient loads which are delivered each year to the NWS (~ 1700 kg N ha^{-1} and ~ 130 kg P ha^{-1}, by considering 365 days of working per year, data not published), but there are some considerations that can give a better understanding of the real contribution of the plants in the nutrient abatement through the NWS.

The first point concerns the development cycle of *P. australis* under natural growing conditions. Since no harvests were foreseen, inevitably plants released nutrients in the litter and residues during fall-winter. About this aspect, from Graneli, 1984 we learn about the reduction of total biomass in *P.australis* from summer to winter of about 28% that could be ascribable to leaves losses. This value was also confirmed by our results since from the following partition among organs registered in summer (leaves: 33.3%; stems: 58.6 %; panicles: 8.1%), in winter we registered in average

1.24% of leaves, 94.88% of stems and 4.36% of flower with a general reduction of total biomass of 5.5 t/ha (Giannini et al., 2016; Giannini et al., in press).

Although the fate of these nutrients is uncertain, it is presumable that they remain immobilized within the organic compounds for long time because of the anoxic condition that the plant residues found on the NWS's bottom when they are not undergone to a chemical-physical stabilization through peat-forming process. Thus, we should add to the amount of nutrient removed by NWS plants also the nutrients that has been taken up in the previous years and translocated in the plant portions fallen on the NWS bottom.

In our case, the renounce of annual harvesting of plants can penalize not only the quantity of biomass and nutrients definitively removed from the system, but also can weaken the reed bed stand. Indeed, the standing dead culms during spring not only intercept light which could be useful for new culms development but it is not usefully used since the leaves, which are the organs photosynthetically active are completely lost (Graneli, 1989). Moreover, many authors report a better reaction of the beds to winter harvests than to summer ones, since the beds have translocated more resources at rhizomes to guarantee a vigorous resprout in the next vegetative season, generally characterized by an increased number of culm per unit of surface (Graneli, 1990; Thompson and Shay, 1985). Conversely, mowing in summer has determined many times an increase of the number of shoots and a reduction of the culms diameter, with different effects on the annual productivity.

Indeed, while some authors reported a decrease in above-ground biomass production after annual summer harvesting (Haslam, 1970; Gusewell et al., 2000), Van den Wyngaert et al. (2003) demonstrate that the production in grazed *Phragmites* could be even higher than the ungrazed one and the nutrient content (nitrogen and phosphorus) of the biomass even doubled.

Thus, if we want to maximize the phyto-treatment potential of the area, we should adopt a more intensive management of plants by mowing *P.australis* every two years during summertime, in this way the high nutrient availability would compensate for the losses without even reducing significantly the life span of the reedbeds (Gusewell et al., 2000).

This type of management, however, by altering the renaturation process could preclude the possibility for the NWS to reaching the climax conditions. Moreover, it is not to neglect that the deposit of the plant residue onto the NWS bottom can contribute to the formation of new peat and so reversing the actual trend of subsidence (Gessner, 2000; Domish et al., 2006) that constitutes a severe issue for the management of lake basin (Silvestri et al., 2017).

Secondly, we have to consider that the overall surface covered by the vegetation (*P. australis* + *M. aquaticum*) is about 1 ha equal only to 38% of the total NWS surface, and if this percentage will increase over time, the nutrient removal can consequently raise. Indeed the nutrients taken up by plants in 4 years of the phyto-treatment system working were about 700 kg of total nitrogen (4 % of the quadriennial nitrogen load) and about 70 kg of total phosphorus (5% of the quadriennial phosphorus load).

In our case study, harvesting during summer 2016 would have meant the removal of the yearly biomass produced by the reedbed during the 4th growing season while the previous yearly productions were lying under the water layer. A gross quantification of the biomass produced during the previous 3 years can be tried multiplying the area covered by *P. australis* by the mean productivity registered during summer 2016, from which we estimate about 120 tons of dry biomass produced and not harvested.

If this biomass presented the same average nutrient content detected in the biomass sampled in 2016, we could estimate about 2 tons of nitrogen and 200 kg of phosphorus taken up by the plant and largely fated to likely stabilization processes.

About *M. aquaticum,* we learnt from Wersal and Maden (2011) the strict connection existing between biomass production and nutrient availability, in particular the biomass is greater with at a high:low N/P ratio, which was also our case (N/P = 10). The negative relationship between yield and P concentration according to Wersal and Maden (2011) could be explained with an increased competition for light and nutrients with algae.

Also in this case, the phyto-treatment ability of this species is significantly enhanced by frequent harvesting (at least annually) (Nuttall, 1985) and we can repeat the considerations already expressed for *P.australis*. In our case study, *M. aquaticum* registered a very high nutrients concentration per unit of dry biomass, which made it competitive with *P.australis* in term of nutrient uptake although the biomass production was definitely lower.

We can estimate a previous productivity of about 11 tons of dry biomass produced and not harvest, containing about 230 kg of total nitrogen and 40 kg of total phosphorus.

Comparing the overall estimated production on four year perspective with the nutrient loads we could access that the phyto-treatment capability of the 'plant system' raised up to 15% for total nitrogen and 20% for total phosphorus.

Given that from the first results on phytotreatment efficiency of the system, we observed a general reduction of total nitrogen of about 30-40% and of total phosphorus of about 50-60%, we can conclude that this system can be profitably used to trap the nutrient.

From a productive perspective, comparing this system with the paludicultural one (PCS) (Giannini et al. in press), we could derive a series of considerations. On the surface unit basis, looking at *P.australis* which is a common species, the NWS seemed to be equally productive to PCS. Indeed, while in PCS we registered a mean yearly yield of 11.5 t/ha in NWS we registered about 49 t/ha after 4 years. However, it is evident that this system cannot sustain a high productivity since the surface covered by vegetation is extremely low and moreover the choice of adopting a longer harvesting turn determine an inconstant level of production over the years. Furthermore, the presence of a consistent water layer (~ 30-35 cm) all-year-long constitutes an obstacle to an annual harvest difficult to be overcome unless to drain periodically the NWS or to use special machines, which generally are not in the availability of farmers.

In both systems, *P.australis* showed similar nitrogen and phosphorus content, confirming that the suitability of this plant is the same in both systems independently on the different hydraulic condition (satured soil for PCS and flooding for NWS).

5. Conclusions and implication for wetland management

The conversion of drained peatlands to natural wetland resulted, under our experimental conditions, an operation quite easy and short to implement. Just few years after the flooding and the partial top soil removal, the re-naturalization process seemed to be well underway as proved by the flora biodiversity increase and the development of some floristic association typical of the surrounding palustrine areas. This fact confirmed the status of peatlands was not as degraded as to prevent the spontaneous vegetation recolonization of the rewetted areas.

The achievement of climax conditions for the NWS can be considered quite close and the fluctuations of the most important vegetation groups (*Phragmitetum* and *Myriophylletum*) were almost determined by the change of local conditions (temperature, height of water level, etc.).

The use of the mixed approach (aerial photos + phytosociological surveys + biomass plant samples) was effective in quantify the cover and productivity of the NWS and is promising in the perspective of monitoring larger natural areas.

In our experimental conditions, characterized by a large availability of nutrients and water both *Myriophyllum aquaticum* and *Phragmites australis* showed remarkable biomass productivity and nutrient taken up, highlighting the phyto-treatment capability of the NWS. The amount of nutrient absorbed by plants during the experimentation period was considerable: about 900 kg N ha^{-1} and 90 kg P ha^{-1} for *P. australis* and about 210 kg N ha^{-1} and 40 kg P ha^{-1} for *M. aquaticum*. However these amount could be considered even larger if we take into account the nutrient present within the portion of biomass fallen each year to the NWs bottom In this case plants would come to abate the 15% and the 20% of the total loads of nitrogen and phosphorous respectively.

To maximize the nutrient abatement capability of NWS, the main factor on which we can act was the choice of harvest strategy. Annual harvesting can increase the amount of nutrients removed by stimulating the plant productivity and taking away from the NWS the biomass produced. At same time, frequent cutting can penalize the peat forming process, make trouble in the management of the harvest operations (necessity of draining area or use of machines able to work in flooded conditions) and cause disturbance to the biota.

For this reason, the management of NWS has to find a compromise able to meet different needs (multifunctional management). At this regard, the authors suggested the strategy of 3-4 year-harvesting-turn occurring in summer. In this way it will be possible to: i) operate longer harvest plans on vegetation communities which are almost at their climax stage, ii) facilitate the deposition of litter and residues of the plants during winter, thus trying to slowing down subsidence, iii) contain the difficulties related to the mechanization of the harvesting.

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Figure captions

Fig.1 Aerial view of the experimental area located in Vecchiano (Tuscany, IT)

Fig.2 Graphical sketch of the pilot field experimental area. CWS: Constructed Wetland System, PCS: PaludiCulture System, NWS: Natural Wetland System which is boxed in red since is the area on which the present paper is focused on.

Fig.3 Long-term (1990-2016) mean monthly temperatures and total rainfall values in Metato (San Giuliano Terme, PI).

Fig.4 Aerial picture of the Natural Wetland System (NWS) with the location of the homogenous units identified for sampling. Areas n. 1, 2, 3, 4, 5, 8 and 9 are of *P. australis* while areas n. 6, 7 and 10 are of *M. aquaticum*.

Fig.5 The diachronic mosaic of the plant cover evolution of the NWS from 2012 to 2016. On left hand, the reconstruction from the ortophotos; on right hand, the sketch reporting the surface covered by each groupment year by year. The analysis reported is referred only to summer flights.

Fig. 6 Fluctuation of the cover levels reached by different groupments from 2013 to 2016.

Table captions

Tab.1 Mean water table levels for the different seasons over the years of observation. The reported values (h in mm) were measured relative to the weir at the outlet of the system.* is used to report period with water flow interruption for operation activities.

Tab.2 Floristic list before the construction works (+ identifies hygrophile species).

Tab.3 Floristic list at June 2016. (* identifies typical hydrophile species; + identifies hygrophile species).

Tab.4 *Phragmitetum australis* (Gams 1927) Schmale 1939. Surveys n. 2, 6, 8 were performed in June 2014; n. 10, 12 were performed in June 2015; n. 13, 14 were performed in June 2016.

Tab.5 –*Typha latifolia* L. groupments. Surveys n. 25 and 26 were performed in June 2014; n. 27 in june 2015.

Tab.6 –*Myriophyllum aquaticum* (Vell.) Verdc. groupments. Surveys n. 28, 29 were performed in June 2015; n. 30 in June 2016.

Tab. 7 – Wet meadows groupments. Surveys n. 31 and 32 were performed in June 2014; n. 33 and 34 in June 2015; n. 35 in June 2016.

Tab.8 - Results of ANOVA on the vegetation parameters. Within each factor, means in the same column followed by different letters are significantly different at $P<0.05$ (Duncan test).

*$p\leq0.05$; **$p\leq0.01$; ***$p\leq0.001$

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Tab.1 Mean water table levels for the different seasons over the years of observation. The reported values (h in mm) were measured relative to the weir at the outlet of the system.* is used to report period with water flow interruption for operation activities.

Mean seasonal value: h (mm)				
	Winter (Dec-Feb)	Spring (Mar-May)	Summer (Jun-Aug)	Fall (Sep-Nov)
2013	50	55	38	65
2014	38*	11*	45	83
2015	76	66	76	70
2016	94	114	84	-

Tab.2 Floristic list before the construction works (+ identifies hygrophile species).

Family	Species	Biological form
Amaranthaceae	*Amaranthus retroflexus* L.	*T scap.*
Asteraceae	*Arctium lappa* L.	*H bienn.*
Asteraceae	*Artemisia verlotiorum* Lam.	*H scap.*
Asteraceae	*Bidens tripartita* L.	*T scap.*
Asteraceae	*Xantium strumarium* L.	*T scap*
Caryophyllaceae	*Silene alba* (Miller) Krause	*H bienn*
Convolvulaceae	*Calystegia sepium* (L.) R. Br.	*H scand +*
Lythraceae	*Lythrum salicaria* L. +	*He (H scap) +*
Malvaceae	*Abutilon theophrasti* Medik	*T scap.*
Phytolaccaceae	*Phytolacca americana* L.	*G rhiz*
Plantaginaceae	*Linaria vulgaris* Mill.	*H scap*
Poaceae	*Bromus tectorum* L.	*T scap.*
Poaceae	*Echinocloa crus-galli* (L.) P. Beauv.+	*T scap +*
Poaceae	*Phragmites australis* (Cav.) Trin. +	*He (G rhiz) +*
Polygonaceae	*Rumex crispus* L.	*H scap*
Solanaceae	*Datura stramonium* L.	*T scap.*
Typhaceae	*Typha latifolia* L. +	*He (G rhiz) +*

Tab.3 - Floristic list at June 2016. (* identifies typical hydrophile species; + identifies hygrophile species).

Family	Species	Biological form
Alismataceae	*Alisma plantago-aquatica L.*	*He (I rad) **
Apiaceae	*Apium nodiflorum (L.) Lag*	*He (I rad) **
Asteraceae	*Eupatorium cannabinum L.*	*H scap +*
Asteraceae	*Artemisia verlotiorum* Lam.	*H scap.*
Convolvulaceae	*Calystegia sepium (L.) R.Br.*	*H scand +*
Cyperaceae	*Carex otrubae Podp.*	*H caesp +*
Cyperaceae	*Cyperus glomeratus L.*	*He (G rhiz) +*
Cyperaceae	*Cyperus longus L.*	*He (G rhiz) +*
Cyperaceae	*Schoenoplectus tabernaemontani (Gmel.) Palla*	*He (G rhiz) +*
Haloragaceae	*Myriophyllum aquaticum (Vell.) Verdc*	*I rad **
Juncaceae	*Juncus articulatus L.*	*He(H caesp)+*
Juncaceae	*Juncus bufonius L.*	*T caesp +*
Juncaceae	*Juncus effusus L.*	*He(H caesp)+*
Lemnaceae	*Lemna minor L.*	*Pl (I nat) **
Lythraceae	*Lythrum salicaria L.*	*He (H scap) +*
Onagraceae	*Epilobium hirsutum L.*	*H scap +*
Poaceae	*Echinochloa crus-galli (L.) P. Beauv.*	*T scap +*
Poaceae	*Phragmites australis (Cav.) Trin.*	*He (G rhiz) +*
Poaceae	*Poa trivialis L.*	*H caesp*
Poaceae	*Polypogon monspeliensis (L.) Desf.*	*T scap+*
Primulaceae	*Samolus valerandi L.*	*He (H scap) +*
Ranuncolaceae	*Ranunculus sceleratus L.*	*T scap*
Typhaceae	*Typha latifolia L.*	*He (G rhiz) +*

Tab. 4 - *Phragmitetum australis* (Gams 1927) Schmale 1939
Surveys n. 2, 6, 8 were performed in June 2014; n. 10, 12 were performed in June 2015; n. 13, 14 were performed in June 2016.

n. survey	2	6	8	10	12	13	14
Surface (m^2)	25	25	25	25	25	25	25
Coverage (%)	100	100	100	100	100	100	100
n. species	6	3	6	6	7	5	5
Phragmites australis (Cav.) Trin.	4	5	5	4	4	4	4
Calystegia sepium (L.) R.Br.	+	+	+	+	+	+	+
Eupatorium cannabinum L.	.	.	.	+	+	+	.
Stachys palustris L.	.	.	+	+	+	.	.
Lythrum salicaria L.	+	.	.	+	+	.	r
Typha latifolia L.	.	r	r	.	.	.	.
Schoenoplectus tabernaemontani (Gmel.) Palla	r	.	.	.	r	.	.
Mentha aquatica L.	.	.	r	.	.	r	.
Iris pseudacorus L.	1	.	.	+	+	.	.
Oenanthe aquatica L.	.	.	+	.	.	.	+
Alisma plantago-aquatica L.	r	.	.	.	.	r	.
Polypogon monspeliensis (L.) Dsf.	.	.	.	.	.	.	r

Tab.5 –*Typha latifolia* L. groupment. Surveys n. 25 and 26 were performed in June 2014; n. 27 in June 2015.

n. survey	25	26	27
Surface (m^2)	9	9	4
Coverage (%)	80	80	100
n. species	4	6	3
Typha latifolia L.	3	2	4
Apium nudiflorum (L.) Lag	.	1	+
Calystegia sepium (L.) R.Br.	+	+	1
Phragmites australis (Cav.) Trin.	1	1	.
Schoenoplectus tabernaemontani (Gmel.) Palla	.	+	.
Lythrum salicaria L.	+	1	.

Tab.6 –*Myriophyllum aquaticum* (Vell.) Verdc. groupment. Surveys n. 28, 29 were performed in June 2015; n. 30 in June 2016.

n. survey	28	29	30
Surface (m^2)	60	100	100
Coverage (%)	80	80	100
n. species	5	5	2
Myriophyllum aquaticum (Vell). Verdc.	5	5	5
Typha latifolia L.	.	r	+
Juncus articulatus L.	+	.	.
Lythrum salicaria L.	+	+	.
Lemna minor L.	+	+	.
Lythrum salicaria L.	+	1	.

Tab. 7 – Wet meadows groupments. Surveys n. 31 and 32 were performed in June 2014; n. 33 and 34 in June 2015; n. 35 in June 2016.

n. survey	31	32	33	34	35
Surface (m^2)	25	25	25	25	25
Coverage (%)	50	70	80	100	100
n. species	10	12	11	7	10
Phragmites australis (Cav.) Trin.	1	+	2	1	2
Echinochloa crus-galli (L.) P. Beauv.	2	1	+	3	2
Calystegia sepium (L.) R.Br.	+	+	+	+	+
Eupatorium cannabinum L.	.	r	.	.	r
Poa trivialis L.	+	.	+	1	.
Juncus effusus L.	.	.	+	.	r
Juncus bufonius L.	.	+	+	.	+
Carex otrubae Podp	r	r	.	..	+
Lythrum salicaria L.	+	+	2	+	r
Schoenoplectus lacustris Palla	+	r	.	.	.
Iris pseudacorus L.	.	r	.	r	+
Paspalum dilatatum Poir.	+	+	+	.	+
Ranunculus scleratus L.		+	+	.	.
Ranunculus sardous Crantz	+	+	+	.	.
Samolus valerandi L.	+	.	.	.	.
Epilobium hirsutum L.	.	.	+	+	.

Tab.8 - Results of ANOVA on the vegetation parameters**.** Within each factor, means in the same column followed by different letters are significantly different at P<0.05 (Duncan test).

*p≤0.05; **p≤0.01; ***p≤0.001

Treatments	Plant species	Biomass (kg/m^2) d.m.	%N	%P	%C
Area 1	*P. australis*	4.77 ab	1.96 ab	0.16 c	44.15 ab
Area 2	*P. australis*	4.77 ab	1.87 ab	0.16 c	45.36 ab
Area 3	*P. australis*	5.01 ab	1.76 ab	0.19 c	45.24 ab
Area 4	*P. australis*	3.15 bc	1.55 b	0.14 c	45.58 a
Area 5	*P. australis*	4.64 ab	1.61 b	0.17c	44.85 ab
Area 6	*M. aquaticum*	0.91 c	2.20 a	0.44 a	39.63 d
Area 7	*M. aquaticum*	1.43 c	1.88 ab	0.30 b	39.70 d
Area 8	*P. australis*	5.75 a	2.03 ab	0.19 c	44.05 b
Area 9	*P. australis*	6.44 a	1.77 ab	0.18 c	44.96 ab
Area 10	*M. aquaticum*	0.80 c	2.05 ab	0.30 b	42.52 c
		***	ns	***	***

Figure1

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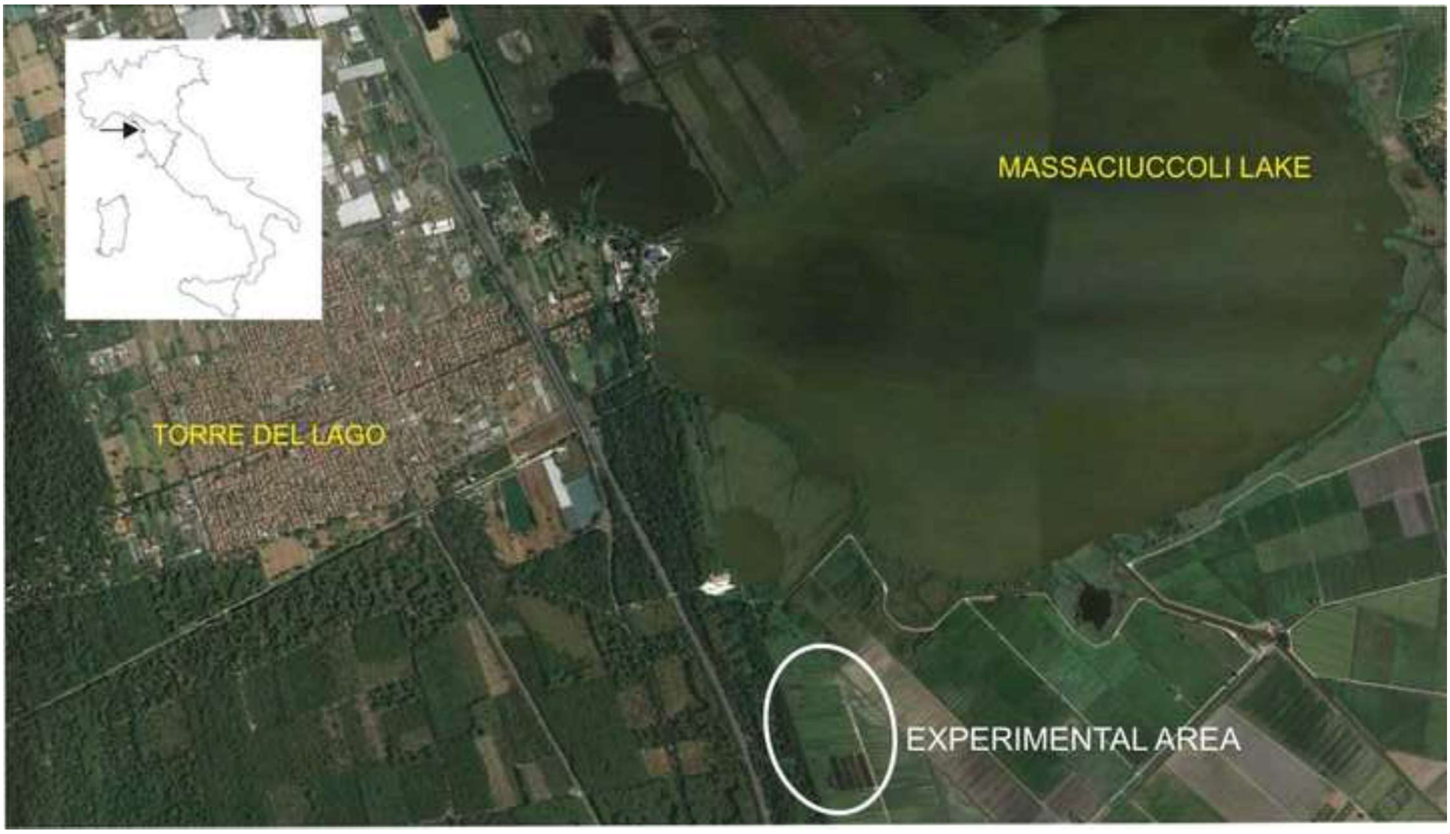

Figure2

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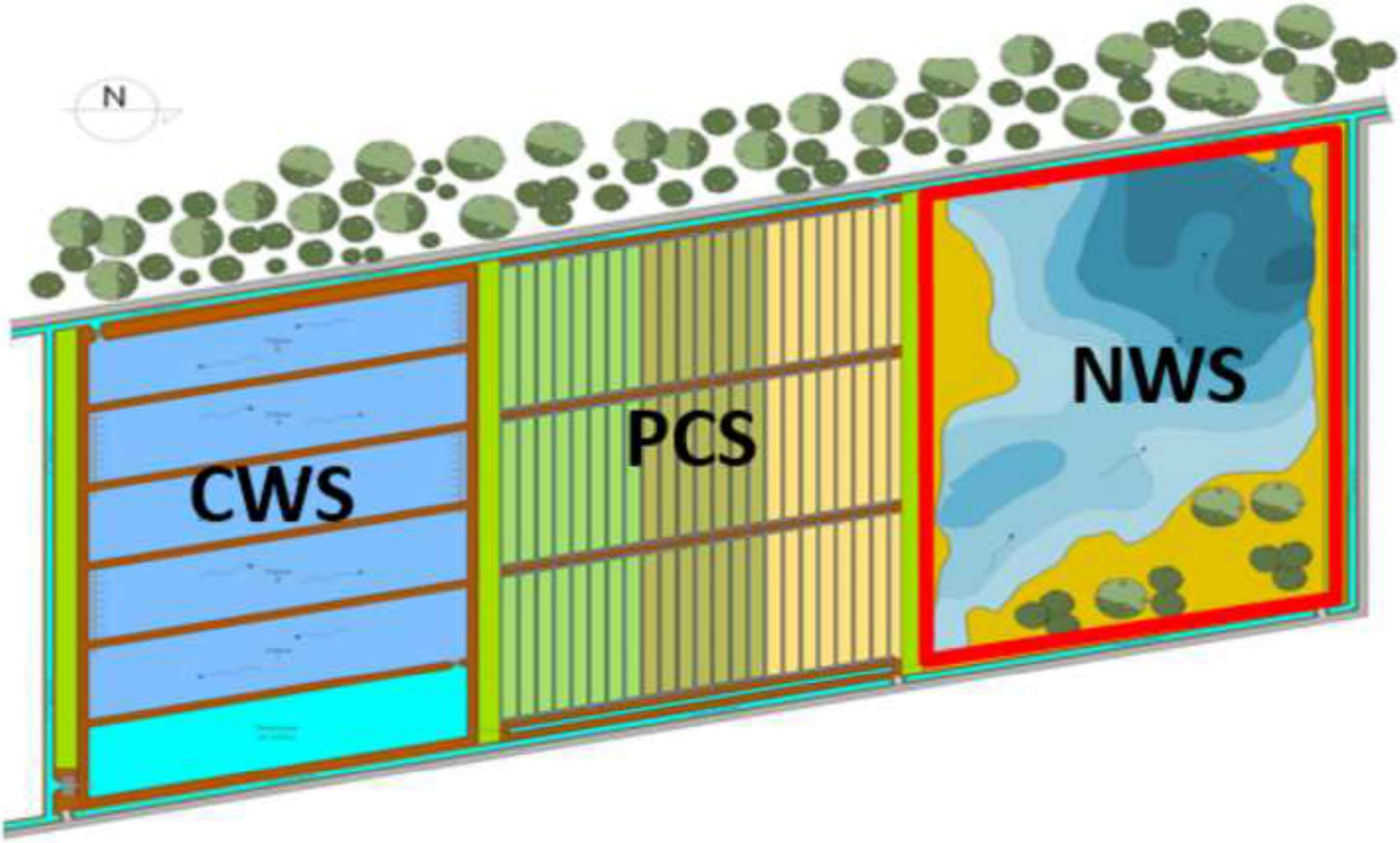

Figure3

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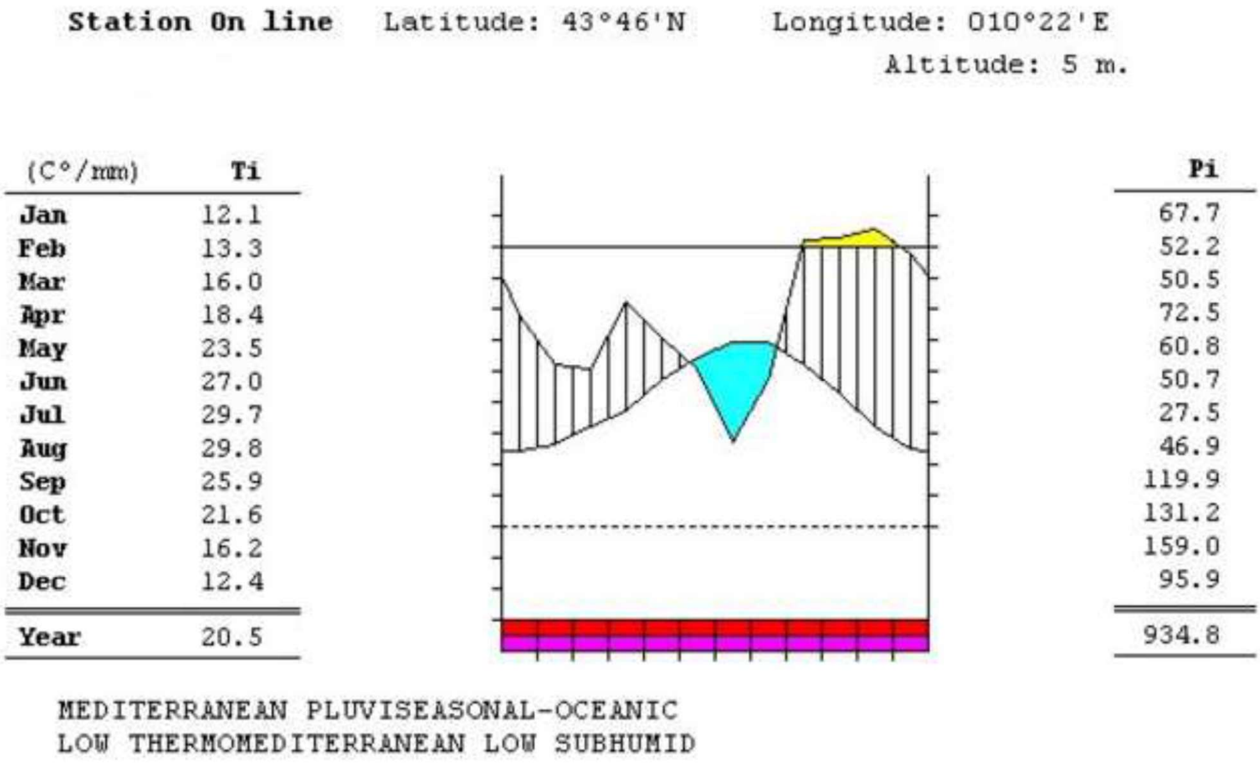

Station On line Latitude: 43°46'N Longitude: 010°22'E
Altitude: 5 m.

(C°/mm)	Ti	Pi
Jan	12.1	67.7
Feb	13.3	52.2
Mar	16.0	50.5
Apr	18.4	72.5
May	23.5	60.8
Jun	27.0	50.7
Jul	29.7	27.5
Aug	29.8	46.9
Sep	25.9	119.9
Oct	21.6	131.2
Nov	16.2	159.0
Dec	12.4	95.9
Year	20.5	934.8

MEDITERRANEAN PLUVISEASONAL-OCEANIC
LOW THERMOMEDITERRANEAN LOW SUBHUMID

Figure4

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Figure5

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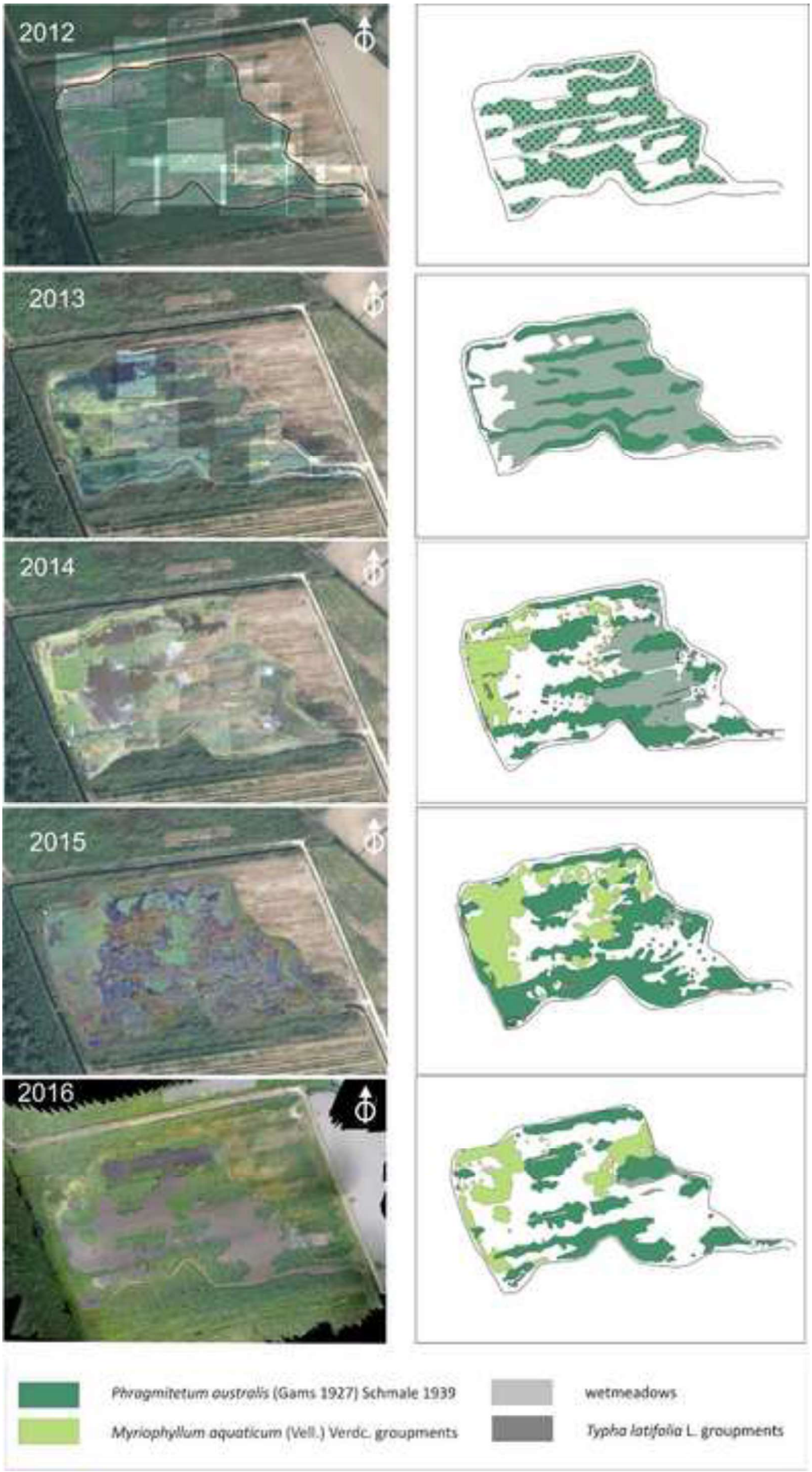

Figure6

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