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Original
Growth and nutrient uptake of perennial crops in a paludicultural approach in a drained Mediterranean peatland / Giannini, V., Silvestri, N., Dragoni, F., Pistocchi, C., Sabbatini, T., Bonari, E.. - In: ECOLOGICAL ENGINEERING. - ISSN 0925-8574. - 103:(2017), pp. 478-487. [10.1016/j.ecoleng.2015.11.049]

Availability:
This version is available at: 11388/230001 since: 2022-05-14T18:54:33Z

Publisher:

Published
DOI:10.1016/j.ecoleng.2015.11.049

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18 November 2026

GROWTH AND NUTRIENT UPTAKE OF PERENNIAL CROPS IN A PALUDICULTURAL APPROACH IN A DRAINED MEDITERRANEAN PEATLAND

Giannini Vittoria[1]*, Silvestri Nicola[2], Dragoni Federico[1], Pistocchi Chiara[1,3], Sabbatini Tiziana[1], Bonari Enrico[1]

[1]Institute of Life Sciences, Scuola Superiore Sant'Anna di Studi Universitari e di Perfezionamento, Piazza Martiri della Libertà, 33- 56127, Pisa (IT)

[2] Department of Agriculture, Food and Environment, Università di Pisa, Via del Borghetto, 80- 56124, Pisa (IT)

[3] Group of Plant Nutrition, Eschikon Experimental Station, ETH Zurich, Eschikon, 33- 8315, Lindau (CH)

* corresponding author: v.giannini@sssup.it

ABSTRACT

Combining peatland rewetting with biomass cropping (paludiculture) is one strategy to remove nutrient surpluses from soil/water and stimulate peat-forming vegetation. This approach was tested in the Massaciuccoli Lake Basin (Tuscany, Italy), a coastal floodplain artificially drained for agricultural purposes since 1930, where land reclamation and continuous cropping have contributed to considerable peat degradation and water eutrophication due to nutrient enrichment of surface waters. An experimental trial was established in spring 2012 with three perennial rhizomatous grasses (PRG) (*Phragmites australis*, *Miscanthus* × *giganteus*, *Arundo donax*) and two woody species managed as short-rotation coppice (SRC) (*Salix alba* 'Dimitrios', *Populus* × *canadensis*

'Oudenberg'), aimed to provide biomass for various bioenergy supply chains. A conventionally cultivated annual crop (maize) was the control. The aim of this study was to compare the sustainability of the proposed paludiculture systems to that of conventional annual crops on the basis of yield and nutrient-removal capability. This 2-year field study evaluated yields, nutrient concentrations and uptake (N and P) of the crops. Over the two years, *A. donax* had the highest mean biomass yield (35 Mg ha^{-1}), N uptake (367 kg ha^{-1}), and P uptake (54 kg ha^{-1}). SRCs had the lowest nutrient uptake in both years. Among grasses, the highest N concentration was recorded in *P. australis* leaves in 2013 (N: 2.41%), while P concentration was greater in *S. alba* branches (P: 0.39%). The average above ground biomass of the maize was 17.5 Mg ha^{-1}, while the nutrient uptakes were equal to 194 and 27 kg ha^{-1} for N and P respectively.

Thus, the performances of paludiculture systems were generally encouraging and could represent an important alternative for restoring and managing former drained peatlands in a suitable product chain.

KEYWORDS: paludiculture, perennial rhizomatous grasses, short-rotation coppice, peatland management, nutrient removal

HIGHLIGHTS

- Three perennial grasses and two woody crops were tested for paludiculture
- All species tested were suitable for paludiculture
- *Arundo donax* had the highest yields and nutrient (N, P) uptakes
- The paludicultural system can be a promising option for peatland management

1. INTRODUCTION

Peatlands are the most widespread wetland types in the world, representing 50-70% of global wetlands. They cover over four million km^2, equal to 3% of the land and freshwater surface of the planet (Joosten and Clarke, 2002), and represent not only a major stock of carbon (C) and nitrogen (N), but also a resource of high ecological, historical, recreational and/or agricultural value (Mitch and Gosselink, 2000).

In previous centuries, many peatlands were artificially drained as a consequence of increasing land demand for agriculture and forestry (i.e. land-hunger) and the urgent need to improve sanitary conditions (i.e. malaria eradication) for the people living there (Holden et al., 2006). As a consequence, significant changes occurred in physical and chemical properties of the peat (Litaor et al., 2008) such as: i) acceleration of organic-matter oxidation, 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 & Wichmann, 2011); ii) increase in NO_3^- concentrations in pore water due to higher oxygen availability and the consequent mineralization and nitrification of organic N (Tiemeyer et al. 2007); and iii) mineralization of organic P compounds and increase of sorbed and Fe-bound P pools (Zak et al. 2004). The continual recurrence of these phenomena has negatively affected the land, for example, progressively lowering the soil level (subsidence), increasing nutrient loads delivered to receiving water bodies (eutrophication) and decreasing ecosystem biodiversity and functionality (loss of ecological stability), especially in land-reclamation districts (Schipper &McLeod 2002; Foley et al. 2005; Tiemeyer et al. 2007; Wichtmann & Joosten, 2007; Verhoeven &Setter 2010). For these reasons, rewetting of drained peatlands has been identified since the mid-1990s as an important mitigation strategy to reverse this self-perpetuating process, which is definitely unsustainable (Erwin, 2009).

73 Restoring saturated conditions reduces GHG emissions from the soil, especially CO_2 and
74 NO_x (Joosten & Augustin, 2006). The use of biomass from plants growing on rewetted
75 peatlands (paludiculture) was evaluated to avoid further CO_2 emissions by replacing raw
76 fossil materials and fuels.
77 Paludiculture is defined as the agricultural use of wet and rewetted peatlands to produce
78 biomass for bioenergy (e.g. direct combustion, biogas, biofuels) or other purposes (e.g.
79 feed; fiber; raw materials for industrial biochemistry, pharmaceuticals and cosmetics),
80 which slows subsidence and nutrient release from the peat soil (reduction of
81 mineralization rate, plant uptake and harvest) and improves ecosystem services (e.g.
82 habitat restoration, aquifer recharge, nutrient cycling) (Wichtmann & Tanneberger 2011;
83 Joosten et al. 2012; Abel et al. 2013; Gunther et al. 2014).
84 In Mediterranean areas, especially in Italy, this approach is quite new because of a
85 historical tradition that always considered the drainage of wetlands (land reclamation) a
86 necessary condition for the development and well-being of human communities.
87 Although peatlands cover less than 1% (about 300 km^2) of Italy's national territory, the
88 vast majority was drained for agricultural purposes in the 1920s-1930s.
89 Our case study is located in a reclamation district on the coastal plain of west-central Italy
90 and is characterized by large-scale, intensive agriculture and the presence of a vulnerable
91 receiving water body, Lake Massaciuccoli. Since the 1930s, a complex network of
92 artificial drains and pumping stations has been used to drain the superficial aquifer and
93 excess rainfall, thus ensuring a water table depth suitable for cultivation (Ciccolini et al.
94 2013). The lake and surrounding marches are Wetlands of International Importance
95 according to the Ramsar Convention since 2014, but their status is seriously harmed by
96 severe eutrophication. Furthermore, the traditional agricultural use of the land-

reclamation district is seriously compromised by increasing difficulties in maintaining the unsaturated zone for crop growth (Zuccarini et al., 2011; Pistocchi et al., 2012). For these reasons, it is necessary to identify suitable alternative management options for this area.

Paludiculture based on perennial species has been tested at the field scale as a possible solution and aims to simultaneously maintain water quality and agricultural use of the land. The perennial nature of these crops dramatically reduces agronomic input requirements (e.g. primary and secondary tillage, seeding, fertilization) compared to those of annual crops, making their cultivation possible in fields not easily accessible due to saturated soil.

Perennial crop productivity should be an important criterion for selecting species for paludiculture. High and steady biomass production, along with high nutrient concentrations, may increase nutrient recovery from the surrounding water, thus reducing eutrophication risk for the receiving water body. Furthermore, high yields may increase farmers' incomes and possibly ensure economic sustainability of the system (Tzanakakis et al., 2009). Increasing fixation of carbon dioxide in organic compounds decreases GHG emissions into the atmosphere and provides more matter to renovate the peat stock, which limits subsidence (Joosten et al., 2014).

The crops selected for our paludiculture experiment, aiming for high biomass production, rapid development and crop hardiness (Bonari et al. 2004; Angelini et al. 2009; Rowe et al. 2009; Mirza et al. 2010), were perennial rhizomatous grasses (PRG) and short-rotation coppice (SRC) crops. The objective of this study was to evaluate the suitability of the selected crops to paludiculture in a drained Mediterranean peatland, on the basis of their above ground biomass production and their nutrients (N and P) uptake. These results were

120 compared with those related to the most cultivated crop in the area (maize), used as
121 control.
122
123 **2. MATERIALS AND METHODS**
124 **2.1 Study site**
125 The research was conducted over two years (2013-2014) on part of a larger
126 phytotreatment system located in Vecchiano, about 10 km from Pisa, Italy (43° 49' 59.5"
127 N; 10° 19' 50.7" E) in the Migliarino-San Rossore-Massaciuccoli Natural Park
128 (http://www.parcosanrossore.it).
129 This 15 ha experimental area was used to compare the efficiency of three different
130 systems in treating the eutrophic drainage water coming from a sub-watershed in the
131 reclamation district. In the area, P has been recognized to be the primary limiting factor
132 for eutrophication and the losses of this nutrient from cultivated fields (dissolved +
133 particulate fractions) are estimated in 2-4 kg ha^{-1} y^{-1} per hectare (Pensabene et al., 1997;
134 Bonari et al. 2013). The three systems have different types of water management (water
135 level and path) and plant management (species, cultivation and harvesting) (Ciccolini et
136 al. 2013). The tested systems were a constructed wetland system (CWS), a natural
137 wetland system (NWS), and a paludiculture system (PCS) (Fig. 1). The last of these was
138 based on growing different non-food crops and harvesting their biomass periodically to
139 ensure nutrient removal from the fields. The system was not dammed and was crossed by
140 a dense network of small channels (about 8 m apart) that supplied both drainage (in
141 autumn and winter) and irrigation (in spring and summer) for the crops through lateral
142 infiltration. The soils of the PCS (Table 1) were classified as Histosol according to the
143 USDA system (Soil Survey Staff, 1975) and as Rheic Histosol according to the FAO

144 system (IUSS, 2006). They are representative of the soils of the land-reclamation district,
145 which are also defined as peat and peaty soils (Pellegrino et al., 2014). The main
146 difference between the PCS and the surrounding areas concerns the water table level. The
147 water table in the watershed is artificially lowered to allow the farmers to cultivate, with
148 noticeable fluctuations during the year (from -0.10 to -0.60 m) depending also on
149 cultivation practices., In the rewetted PCS the water table depth is kept higher because of
150 the continuous supply of water to be treated and thanks to the weirs, that are not moved
151 except for management needs (e.g. harvest or maintenance of drainage ditches). Then, in
152 the experimental fields the water table depth is only dependent on the meteorological
153 conditions (e.g. rainfalls or dry periods), ranging from 0.00 to -0.05 m during the winter
154 and from - 0.10 to -0.25 m during the summer. The climate is Mediterranean (Csa)
155 according to the Köppen–Geiger climate classification map (Kottek et al., 2006).
156 Summers are dry and hot, rainfall is mainly concentrated in autumn and spring (mean
157 annual rainfall = 945 mm) and mean monthly air temperature at 2 m ranges from 7°C in
158 February to 30°C in August (mean = 14.8 °C). Mean monthly temperatures and rainfall
159 for 2013-2014 and over the long term (1989-2014) were recorded at a weather station
160 located in the Massaciuccoli basin (Fig. 2).
161
162 **2.2 Trial set up**
163 The experimental design was a completely randomized design with the crop as treatment
164 and three replicates represented by the field sections between two adjacent channels (8 m
165 × 300 m = 2400 m^2). Five species were used in the PCS: i) *Populus* x *canadensis* Moench.
166 var. "Oudemberg" (Pop) and *Salix alba* L. var. "Dimitrios" (Sal), belonging to the SRC
167 group; and ii) *Arundo donax* L. (Aru), *Miscanthus* × *giganteus* Greef et Deuter (Mis), and

168 *Phragmites australis* L. (Phr), belonging to the PRG group. Maize (*Zea mays* L.) was the
169 annual crop (AC) used as the control; it is the most widespread crop in this area, cultivated
170 for grain production in continuous cropping or two-year rotations with winter wheat or
171 sunflower (Silvestri et al., 2012). Maize was cultivated in the fields surrounding the
172 phytotreatment system under rainfed conditions, since the shallow water table supplied
173 sufficient water to the crop. Two maize cultivation purposes were considered: grain
174 production (Maize-gr) and whole-plant harvest (Maize-wp).
175 Before planting, the land was uncultivated and dominated by spontaneous vegetation
176 (Pellegrino et al., 2014). Crops were established in spring 2012; however, the first year
177 of cultivation (2012) was not assessed because the phytotreatment system did not operate
178 until January 2013.
179 Tillage was performed in autumn 2011 by plowing, followed by rotary harrowing
180 immediately before planting. Mis and Phr were planted at a density of two rhizomes per
181 m^2 (1.0 x 0.5 m spacing, 20,000 rhizomes per ha), while Aru was planted using pre-rooted
182 plants at the same density as that of the other PRGs. The SRCs Sal and Pop were
183 established using one-year-old dormant cuttings (2.0 x 0.75 m spacing, 6,600 plants per
184 ha).
185 The maize agronomic management (Table 2) reflected the practices usually adopted by
186 the farmers in this area. No fertilizers or pesticides were applied to the other crops. At
187 planting, the weed control was mechanical for SRCs (until machines could gain access to
188 the inter-rows) and manual for PRGs. Irrigation was provided during the first growing
189 season, from establishment to October 2012, to supply water until the phytotreatment
190 system began operating. The harvest was annual for PRGs and bi-annual for SRCs.
191

192 **2.3 Data collection and processing**

193 Aboveground biomass yield of each crop was measured in both years of the experiment
194 (2013 and 2014). The PRGs and AC were harvested in late September-early October,
195 while SRCs were collected in December. For PRGs and AC, samples were obtained by
196 pooling three sub-samples (2 m^2) for each field replicate. For each sample, the number of
197 plants and the biomass fresh weight were recorded. For PRGs and AC, 10 stems and 4
198 plants, respectively, were partitioned into leaves, stems and panicles or grains, and the
199 fresh weight of separated parts was determined. SRC plant density was measured over a
200 30 m linear distance. Next, three representative transects of six consecutive plants were
201 harvested for each replicate. Leaves and branches were separated from the stem, and the
202 fresh weights were recorded for the overall sampled biomass and the different plant parts.
203 Yield per ha was calculated as a function of observed plant density. Afterwards, all
204 subsamples were dried at 60°C until a constant weight to determine the dry matter
205 contents of all plant components and the dry matter yields of the crops (Mg ha^{-1}). The
206 dried samples were ground to 1 mm and used for tissue-nutrient analyses following
207 digestion of 200 mg of plant material by H_2SO_4/H_2O_2 (Bremner 1965). N concentration
208 was determined with the Kjeldahl method, while P concentration was determined with
209 the molybdenum blue method using a Perkin Elmer Lambda 25 spectrophotometer
210 (Giannini et al., 2015). Nutrient uptake (kg ha^{-1}) for each plant part was calculated as the
211 product of its nutrient concentration and dry biomass weight. The overall nutrient uptake
212 of each crop was calculated as the sum of the uptake of each part.
213 As reported in chapter 2.2, data were analyzed according to a completely randomized
214 design, with the crop as treatment replicated three times. When significant differences
215 were observed, means of different plant species were compared using Tukey's honest

significant difference (HSD) post-hoc test at the 0.05 p-level. All statistical analyses were performed with R statistical software (version 3.1.1, R Foundation for Statistical Computing).

3. RESULTS

3.1 Plant density and harvestable production

The plant densities surveyed in PRG and AC areas and on SRC transects were consistent with the hypothetical values resulting from the distances of seeding or planting.

In general, PRGs had higher values of harvestable production than the two other groups, which were similar to each other, although slightly higher for the AC (Fig. 3). This pattern was the same in 2013 and 2014.

Among individual species, mean yield over the two years was highest for Aru (36. 6 Mg ha^{-1}), followed by Mis (24.7 Mg ha^{-1}) and Maize-wp (17.5 Mg ha^{-1}). Phr (11.5 Mg ha^{-1}) and Pop (8.4 Mg ha^{-1}) yielded lower amounts of biomass, with not statistically different results in 2013 and in 2014. Sal (7.1 Mg ha^{-1}) had the lowest harvestable yields in 2013 but had yields similar to those of Phr, Pop, and Maize-gr in 2014.

The species in the SRC group showed a different pattern during the experiment. Pop was significantly more productive than Sal in 2013, while Pop and Sal had similar yields in 2014. The mean harvestable production of Maize-gr (8.8 Mg ha^{-1}) was similar to that of Pop in 2013 and to those of all SRCs in 2014.

3.2 Nutrient concentration

Nutrient concentrations in the tissues of different species varied greatly depending on the plant part analyzed (leaves, stems, branches, panicles, grain, etc.) and were also

affected by the experiment year (Table 3). The highest variability in whole-plant nutrient concentration was found for P: coefficients of variability were high for Aru 2013 (22%), Pop 2013 (29%), Mis 2014 (43%), Pop 2014 (30%), and Sal 2014 (23%). Conversely, coefficients of variability of whole-plant N concentrations were higher than 20% in only one case (Aru 2014).

The highest N concentrations (calculated as weighted means) of harvestable plant parts were for Phr and Maize-gr , while Sal had the highest P concentrations (). Mis had low concentrations of both N () and P (). Among plant parts, the highest nutrient concentrations were found for N in the leaves of Aru () and for P in the branches of Sal.

3.3 Plant N uptake

Over the two years, Aru had the highest mean N uptake (367 kg ha^{-1}), followed by Maize-wp (194 kg ha^{-1}), which was not statistically different from Mis and Phr in 2013. N uptake of Mis (mean = 151 kg ha^{-1}) greatly decreased in 2014 (from 207 kg ha^{-1} in 2013 to 94 kg ha^{-1} in 2014) because of a corresponding decrease in N concentration (from 0.78% to 0.42%) (Fig.4). Phr had an inverse pattern, in which N uptake increased in 2014 due to greater harvestable production (+6.80 Mg ha^{-1}). Mean N uptake of Maize-gr (134 kg ha^{-1}) was slightly lower and similar to that of Phr in 2013.. Sal and Pop had the lowest mean N uptake (45 and 38 kg ha^{-1}, respectively) and were significantly different from those of all other species, except for Mis in 2014. Mean N concentrations of PRGs (1.06%) were higher than those of SRCs (0.60%); thus, the pattern of N removal was similar to that of harvestable production, which widens the gap between the two groups of plants. Mean maize N concentrations were the highest (Maize-gr = 1.52% and Maize-wp = 1.09%),

263 which made its N uptake more similar to those of PRGs and consequently increased
264 differences with performances of the SRCs.
265
266 **3.4 Plant P uptake**
267 Over the two years, Aru had the highest mean P uptake (54 kg ha^{-1}); however, Mis P
268 uptake was higher in 2013 (43 vs 33 kg ha^{-1}) because of the low P concentration in Aru
269 biomass (0.09%). Mean P uptake by Maize-wp and Maize-gr was even lower (27 and 20
270 kg ha^{-1}, respectively), while those for the other species (Sal, Phr, and Pop) were similar
271 to each other (18-20 kg ha^{-1}) (Fig. 5).
272 The pattern of P uptake was different for the two years. In 2013, Mis and Aru had
273 significantly higher P uptake than the other species.. In 2014, Aru and Sal had the highest
274 and lowest P uptake, respectively, while those of the other species were not statistically
275 different. Maize-wp = Maize-gr).
276 Mean P concentration was higher in SRCs (0.27%) than in PRGs (0.15%), leading to a
277 smaller relative difference in P uptake compared to N uptake. Mean P concentration of
278 Maize-gr (0.22%) layed between those of PRGs and SRCs, while that of Maize-wp
279 (0.15%) was the same as those of PRGs. This led to a general flattening of the values of
280 P uptake, particularly in 2013 (max = 43 and min = 5 kg ha^{-1} for Mis and Sal,
281 respectively), which was also noticeable in 2014 (max = 74 and min = 20 kg ha^{-1} for Aru
282 and Pop, respectively).
283
284
285 **4. DISCUSSION**

286 To assess the suitability of different species, it is important to evaluate their adaptability
287 and productive capacity under paludicultural conditions. Adaptability is inferred by
288 comparing yields in the paludiculture system with those obtained under similar conditions
289 (i.e. climate, growing season) in unsaturated soils (intraspecific assessment), while
290 productive capacity is evaluated by comparing harvestable productions of the species
291 under paludicultural conditions to those of traditional annual crops (interspecific
292 assessment).
293 All the PRGs tested showed good adaptability to tested conditions. Mean yields of Aru
294 and Mis over the two years, corresponding to their 2nd and 3rd growing seasons, were 36.5
295 and 25.0 Mg ha^{-1}, respectively, which are similar to those obtained at the same crop age
296 in a mineral soil with a lower water table level in the same area (the coastal plain of
297 western-central Italy) (Angelini et al., 2005, 2009; Roncucci et al., 2014). Harvestable
298 yields of Aru were also similar to those obtained in southern Italy by Borin et al. (2013)
299 in mineral soils irrigated with nutrient-rich water (37 and 29 Mg ha^{-1} in the 2nd and the 3rd
300 growing seasons, respectively). In contrast, Mis yields observed in the same area by
301 Angelini et al. (2009) were slightly higher (48 and 29 Mg ha^{-1} in the 2nd and the 3rd
302 growing seasons, respectively). Therefore, cultivation of Aru and Mis in a paludicultural
303 cropping system did not seem to penalize their potential productivity, and they adapted
304 well to rewetting conditions in the two years considered.
305 The production of Phr is more difficult to evaluate because of the lack of experiments in
306 the same area. However, despite lower yields than the other PRGs, Phr was considered
307 adaptable by several authors, since it typically grows under wetland conditions (Graneli,
308 1984; Egloner, 2009; Wichtmann and Couwenberg, 2013). Comparison with other Italian
309 experiments highlights that the mean yield of Phr in this study (11.5 Mg ha^{-1}) was higher

than those reported by Molari et al. (2014) on four sites, in which biomass production ranged from 2.3-8.5 Mg ha^{-1} on mineral soils irrigated with nutrient-rich waters simulating agricultural-drainage effluents. Köbbing et al. (2013) reported yields ranging from 5-20 Mg ha^{-1} in northern Europe and China. Hence, in this study Phr had biomass yields in line with those reported in the literature for this species.

The productivity of woody crops (SRCs) depend greatly on the varieties selected, as well as on soil and environmental conditions in which the species are grown, thus making comparisons among experiments more difficult. At the first cut, a hybrid *Populus* clone, grown in the same coastal plain in Italy at a higher density (10,000 plants ha^{-1}) on a mineral soil managed with a 2-year SRC cycle had a mean yield of about 45 Mg ha^{-1}, which corresponds to a mean of 22.5 Mg ha^{-1} y^{-1} (Nassi o di Nasso et al., 2010). The yield recorded in 2014 for Pop (10.3 Mg ha^{-1}), having its roots in the 3rd growing season and one-year-old aboveground organs, was similar to the long-term mean reported by the same study for hybrid poplars (*Popolus* sp.) cut every year (9.9 Mg ha^{-1}). Regarding willow (*Salix* sp.), the available data collected in the same area are related to lysimeter experiments on an unfertilized mineral soil, which showed a yield of 6.6 Mg ha^{-1} in the 2nd growing season, which corresponds to 3.3 Mg ha^{-1} y^{-1} (Guidi et al., 2008). Typical biomass yields of willow and poplar in European climates range from 3-12 Mg ha^{-1} y^{-1} (Kauter et al., 2003; Keoleian & Volk, 2005), while maximum yields under optimal conditions can reach 28-30 Mg ha^{-1} y^{-1} (Don et al., 2012). However, it should be noted that biomass yields reported in the literature depend greatly on the experimental set-up. Higher yields are generally obtained from experiments with small plots, and therefore significant edge effects, while lower yields are reported for large plots and open-field cultivation (Kauter et al., 2003). Overall, under our experimental conditions, interesting

productivity was observed in Pop and Sal during the 3rd growing season, despite having modest yields in the 2nd growing season.

Maize showed no relevant differences in yield between the two years, as similar and remarkable harvestable yields were observed (Maize-gr: 8.2 and 9.4 Mg ha^{-1} in 2013 and 2014, respectively; Maize-wp: 16.2 and 18.8 Mg ha^{-1} in 2013 and 2014, respectively). This pattern indicated that maize, as observed by Silvestri et al. (2012),can achieve high productivity under the hydraulic conditions present in the reclamation district, characterized by a shallower water depth compared to other areas in the region. Comparison with yields obtained in the same area shows that productivity of the rainfed maize was strongly affected by water availability. Maize subject to a shallow water table had whole-plant yields in line with our results (8-16 Mg ha^{-1}) (Bellocchi et al., 2002), while a deeper water table led to lower yields (6-10 Mg ha^{-1}) (Mazzoncini et al., 2011).

Comparison of PRG and SRC species with the AC established a clear ranking according to their harvestable production. Although cultivation conditions (nutrients and water availability) were quite steady and temperature did not vary greatly between the two years, some variations were observed in harvestable yields. From the 2nd to the 3rd growing season, biomass yields of Aru and Mis slightly decreased (-9.5% and -15%, respectively), which could be consequence of prolonged soil saturation. Although Mann et al. (2013) reported acceptable performances of the two species under both flooded and field-capacity conditions, Lambert et al. (2014) highlighted that Aru produced about 50% less biomass in flooded cultivation. Both species were classified as moderately tolerant to extreme moisture conditions (Quinn et al., 2015).

Conversely, willow and poplar showed increasing yields (62% and 830%, respectively), probably in relation to the progressive development of their root systems, with a

358 consequent increase in production capability (Wilkinson, 1999). Phr also had higher
359 harvestable biomass in 2014 than in 2013 (+84%), which could be explained by the higher
360 shoot density in the second year (data not shown). Maize (Maize-gr and Maize-wp)
361 showed no noticeable difference in yield between the two years (+14% for 2014), in
362 relation to its annual crop cycle and to the agronomic practices provided each year (Table
363 2).
364 Due to the harvestable yield trends that might be observed in future years, it is still not
365 possible to identify the most suitable crop for this paludicultural system. Therefore, the
366 experimental period should be extended for several years to evaluate crop behavior at a
367 steady-state, after crops have spent a few years under saturated soil conditions and have
368 reached maturity. Moreover, the sustainability of the paludiculture option also depends
369 on the environmental aspects and on the profitability of peatland management systems.
370 In particular, aiming to decrease nutrient concentrations in the water to be treated, the
371 assessment of nutrient-uptake rates is crucial to maximize the benefits of crop use.
372 Especially in the case of P, the removal of the nutrient from the water-soil system by
373 harvesting represent an important path to cut down the loads delivered to the receiving
374 water body. Regarding N, the contribution of plant uptake to reduce the transfer to water
375 bodies can be less important, considering the gaseous losses from the soil, such as
376 denitrification and/or production of nitrogen oxides (Järveoja et al., 2015).
377 In our study, mean amounts of nutrients removed were high for Aru (367 kg N ha^{-1} and
378 54 kg P ha^{-1}) and notable for Mis (151 kg N ha^{-1} and 37 kg P ha^{-1}) and Phr (172 kg N ha^{-1}
379 and 19 kg P ha^{-1}). These quantities are of the same order of magnitude as the nutrient
380 loads carried to the PCS by the water (up to 350 kg N and 25 kg P ha^{-1} y^{-1} if the water-
381 treatment system operates 365 days per year), indicating that plant uptake can remove a

382 significant portion of the nutrients introduced into the system. In Aru, about 68% of N
383 and 30% of P was contained in the leaves, thus highlighting their relevant role at harvest
384 in nutrient removal by this species. Conversely, concentrations in leaves were lower in
385 the other PRGs, mostly due to a lower leaf-mass ratio (Mis) and to higher nutrient
386 concentrations in the stems (Phr). This can have practical implications in timing crop
387 harvests. The species in which foliar mass plays a relevant role in uptake and early
388 harvests are tolerated (i.e. Aru) could be harvested before senescence, thus preventing
389 leaf loss and increasing nutrient removal (Dragoni et al., 2015). However, the lower
390 contribution of the leaves of Mis to overall uptake and lower tolerance of early cuts
391 (Strullu et al., 2013) means that it could benefit from delayed harvests. In turn, harvest
392 timing has potential effects on nutrient relocation to belowground organs and thus on the
393 lifespan of PRG plantations (Lewandowski et al., 2003).
394 Regarding Pop and Sal, the amounts of nutrients removed are lower than those of other
395 crops (Pop: 38 kg N ha^{-1} and 18 kg P ha^{-1}; Sal: 45 kg N ha^{-1} and 19 kg P ha^{-1}) and their
396 leaves represent a large portion of the plants that cannot be collected, since most fall to
397 the ground during conventional harvest times (autumn-winter). On the other hand, early
398 harvests on these species are not advisable, since they can greatly reduce the lifespan of
399 the crop stands (Sennerby-Forsse et al., 1992). Therefore, most leaves are excluded from
400 nutrient removal, although they can temporarily immobilize non-negligible amounts of
401 nutrients and help build peat stocks. As reported by Tzanakakis et al. (2009), the quantity
402 of leaves produced by *Populus* sp. in the 2nd and 3rd growing seasons was about 8-9% of
403 total accumulated biomass, which equals a temporary uptake of 17-20% and 11-15% of
404 the total N and P, respectively, taken up by the plant. An analogous contribution to C and
405 nutrient immobilization can be envisaged for maize stover left in the field when only grain

406 is collected (Maize-gr). Moreover, maize had relatively high nutrient uptake, particularly
407 when the entire plant was harvested (Maize-wp: 194 kg N ha^{-1} and 27 kg P ha^{-1}; Maize-
408 gr: 134 kg N ha^{-1} and 20 kg P ha^{-1}).
409 Although in the present study the belowground portion of biomass was not investigated,
410 the knowledge about the quantity and distribution of roots is important to better
411 understand the crop effect on soil organic matter accumulation, nutrients uptakes and C
412 sequestration (Monti e Zatta, 2009; Nassi o Di Nasso et al., 2013; Roncucci et al., 2013).
413 Moreover, several studies carried out in Europe highlighted the relevant contribution of
414 vegetation on mitigation of CO_2, CH_4 and N_2O emissions from cultivated peatlands
415 compared to abandoned peat soils (Gunther et al., 2014; Järveoja et al., 2015; Mander et
416 al., 2015).
417 All these results considered, it should be highlighted that maize cultivation also involves
418 fertilizer inputs, making this crop even less effective from an environmental point of view
419 when compared with paludicultural options. Furthermore, ceasing agricultural activities
420 will not significantly reduce the annual rate of soil organic matter mineralization
421 (Pellegrino et al., 2014), and only prolonging saturated conditions in the soil can slow
422 down the chemical and physical subsidence (Wichtmann and Wichmann, 2011).
423 Achieving these conditions represents the main objective in managing the degraded
424 peatland, and paludiculture can combine two difficult issues: i) maintaining agricultural
425 use of a reclamation district and ii) affordably slowing down peat degradation. Despite in
426 Italy peatlands cover limited areas (300 km^2), the improvement of their management is a
427 priority from both economic and environmental point of view. For this reason the
428 involvement of the local stakeholders and farmers, that are reluctant until now, becomes

fundamental in order to gain a shared and conscious knowledge about paludiculture which represents an alternative for peatlands, people and rural economies.

Indeed, economic sustainability of paludiculture systems and the willingness to change of the farmers, are essential prerequisites for its spread in the territory. Considering the yields discussed in this paper, we can derive that under current market conditions, the expected gross production of the AC (revenues = yield x selling price of grain) would be reached by SRCs and PRGs at selling prices for biomass up to 3-4 times lower than that of maize, considered the higher yields gained from these crops (in particular PRGs). Therefore the profitability (revenues – costs) of PCS seems related to the limiting of cultivation cost, in particular those of two specific work-chains: planting and harvesting.

5. CONCLUSIONS

Restoration of degraded peatland represents an important issue at both local and global scales. Agricultural use of peatland based on traditional drainage and cropping systems is becoming progressively less adequate, and new management strategies should be defined. Paludiculture may represent an option that preserves agricultural use of land and reverses peatland degradation, which is particularly intense in Mediterranean conditions.

The findings of the present study demonstrate the agronomic suitability of the crops tested and showed some of the environmental benefits achievable, although a need exists for a longer experimental period to reach steady-state conditions. Results revealed the great influence of species on biomass yields and nutrient uptake, which represent important points for paludiculture system design, along with other aspects that should be investigated (e.g. harvest time, water table level control, lifespan). The choice of an adaptive approach (paludiculture) in peatland management instead of a transformative

453 approach (land reclamation) would require an important change in attitude in land
454 planning, agronomy and farming.
455
456 **ACKNOWLEDGMENTS**
457 This work was supported by the “Consorzio di Bonifica Versilia-Massaciuccoli” and
458 funded by the “Regione Toscana”. The authors wish to thank the technical staff of the
459 Consorzio di Bonifica Versilia-Massaciuccoli for managing the experimental site and
460 harvesting biomass from the entire experimental pilot field, and Fabio Taccini and Sergio
461 Cattani for their help sampling and milling biomass.
462
463

FIGURE CAPTIONS

Fig. 1: Aerial view of the experimental pilot field represented by three different management systems: constructed wetland system (CWS), paludiculture system (PCS) and natural wetland system (NWS). The conventionally drained area cultivated with annual crops is near the pilot field.

Fig. 2: Long-term mean monthly rainfall and temperatures (1989-2014) and monthly rainfall and mean air temperatures in 2013 and 2014 in Vecchiano (Pisa, Italy). The chart is presented as a Bagnouls & Gaussen (1957) diagram to identify dry months, i.e. when the value of rainfall (P, in mm) does not exceed twice the value of mean air temperature (T, in °C) ($P \leq 2T$).

Fig. 3: Crop yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus* x *giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Pop,: *Populus* x *canadensis*; Sal, *Salix alba*) and of annual crop (AC: Maize-wp, maize whole-plant harvest; Maize-gr, maize grain production). Significance of differences according to one-way ANOVA is reported (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$). Vertical bars are the mean standard deviations. Different combinations of lower case letters indicate significantly differing means ($p < 0.05$, Tukey's test).

Fig. 4: Nitrogen uptake and allocation in different plant parts yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus* x *giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Pop,: *Populus* x *canadensis*; Sal, *Salix alba*) and of annual crop (AC: Maize-wp, maize whole-plant harvest; Maize-gr, maize grain production). Black: leaves (PRGs), leaves+branches (SRCs); dark gray: stems (PRGs, SRCs); light gray: panicles (PRGs); diagonal hash marks: maize (AC). Significance of differences according to one-way ANOVA is

reported (*p≤0.05; **p≤0.01; ***p≤0.001). Vertical bars are the mean standard deviations. Different combinations of lower case letters indicate significantly differing means ($p < 0.05$, Tukey's test).

Fig. 5: Phosphorus uptake and allocation in different plant parts yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus* x *giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Pop,: *Populus* x *canadensis*; Sal, *Salix alba*) and of annual crop (AC: Maize-wp, maize whole-plant harvest; Maize-gr, maize grain production). Black: leaves (PRGs), leaves+branches (SRCs); dark gray: stems (PRGs, SRCs); light gray: panicles (PRGs); diagonal hash marks: maize (AC). Significance of differences according to one-way ANOVA is reported (*p≤0.05; **p≤0.01; ***p≤0.001). Vertical bars are the mean standard deviations. Different combinations of lower case letters indicate significantly differing means ($p < 0.05$, Tukey's test).

TABLE CAPTIONS

Table 1: Physical and chemical characteristics of paludiculture system soils (0-30 cm depth).

Table 2: Main maize cultivation practices for grain production (Maize-gr) and whole-plant harvest (Maize-wp).

Table 3: Mean plant-tissue nutrient contents of plant species in the 2013 and 2014 growing seasons. Aru: *Arundo donax*; Mis: *Miscanthus* x *giganteus*; Phr: *Phragmites australis*; Pop: *Populus* x *canadensis*; Sal: *Salix alba*; Maize-wp, maize, whole plant; Maize-gr, maize, grain harvest. Standard deviations are reported in brackets.

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711

712 Table 1. Physical and chemical characteristics of paludiculture system soils (0-30 cm depth).
713 *atomic absorption; **extractable with ammonium oxalate.

Parameter	**Unit**	**Value**
pH		5.0
EC	(dS m^{-1})	1.46
sand (USDA)	(%)	56
silt (USDA)	(%)	25
clay (USDA)	(%)	19
bulk density	(g cm^{-3})	1.44
SOM (Walkey-Black)	(%)	30.1
N_{tot} (Kjeldahl)	(g kg^{-1})	13.2
P_{avail} (Olsen)	(mg kg^{-1})	79
K_{exch}*	(g kg^{-1})	516
CEC	(meq $100g^{-1}$)	75
Fe* *	(g kg^{-1})	12.2
Al* *	(g kg^{-1})	5.5

714

715 Table 2. Main maize cultivation practices for grain production (Mgr) and whole-plant harvest
716 (Mwp). * urea (sowing time), ammonium nitrate (top dressing); ** triple superphosphate; ***
717 potassium sulphate.

Operation	Mgr	Mwp
main tillage	plowing (25-30 cm deep)	
nitrogen fertilization*	160 kg ha^{-1} of N (sowing time) 80 kg ha^{-1} of N (top dressing)	200 kg ha^{-1} of N (sowing time) 100 kg ha^{-1} of N (top dressing)
phosphorus fertilization**	90 kg ha^{-1} of P_2O_5	120 kg ha^{-1} of P_2O_5
potassium fertilization***	60 kg ha^{-1} of K_2O	90 kg ha^{-1} of K_2O
chemical weed control	post-emergence	
seeding rate	7.3 seeds per m^2	8.3 seeds per m^2
maize hybrid	FAO class 600	
harvest	October	September
residue management	chopping and soil mixing at plowing	removal at harvest

718

Table 3: Mean plant-tissue nutrient contents of plant species in the 2013 and 2014 growing seasons. Aru: *Arundo donax*; Mis: *Miscanthus* x *giganteus*; Phr: *Phragmites australis*; Pop: *Populus* x *canadensis*; Sal: *Salix alba*; Maize-wp, maize, whole plant; Maize-gr, maize, grain harvest. Standard deviations are reported in brackets.

Species	Plant part	2013			2014		
		% of total biomass	%N	%P	% of total biomass	%N	%P
Aru	Leaves	31.7 (1.5)	2.43 (0.10)	0.07 (0.03)	27.0 (3.1)	2.16 (0.68)	0.21 (0.01)
	Stems	68.3 (1.6)	0.56 (0.07)	0.09 (0.02)	73.0 (3.0)	0.39 (0.06)	0.22 (0.01)
	Panicles	-	-	-	-	-	-
	Whole plant	-	1.15 (0.06)	0.09 (0.02)	-	0.86 (0.20)	0.21 (0.01)
Mis	Leaves	21.1 (1.9)	1.27 (0.12)	0.11 (0.04)	17.2 (0.9)	0.79 (0.01)	0.10 (0.04)
	Stems	74.5 (2.0)	0.60 (0.08)	0.17 (0.05)	78.7 (1.4)	0.31 (0.04)	0.14 (0.07)
	Panicles	4.3 (0.1)	1.58 (0.21)	0.38 (0.10)	4.1 (0.5)	0.86 (0.10)	0.25 (0.04)
	Whole plant	-	0.78 (0.11)	0.16 (0.03)	-	0.42 (0.03)	0.14 (0.06)
Phr	Leaves	33.3 (1.6)	2.41 (0.28)	0.16 (0.03)	33.2 (1.7)	1.85 (0.17)	0.24 (0.01)
	Stems	58.6 (1.8)	1.63 (0.23)	0.12 (0.02)	57.6 (2.5)	1.15 (0.16)	0.18 (0.02)
	Panicles	8.1 (0.1)	0.76 (0.17)	0.07 (0.01)	9.2 (1.9)	0.53 (0.10)	0.09 (0.01)
	Whole plant	-	1.82 (0.21)	0.13 (0.02)	-	1.33 (0.06)	0.19 (0.01)
Pop	Leaves + Branches	52.8 (6.0)	0.45 (0.09)	0.29 (0.05)	14.4 (6.0)	0.86 (0.08)	0.27 (0.08)
	Stems	47.2 (5.9)	0.52 (0.02)	0.19 (0.08)	85.6 (6.0)	0.34 (0.08)	0.19 (0.05)
	Whole plant	-	0.48 (0.05)	0.24 (0.06)	-	0.42 (0.04)	0.20 (0.06)
Sal	Leaves + Branches	24.5 (3.4)	1.44 (0.27)	0.36 (0.06)	27.4 (5.2)	0.94 (0.04)	0.39 (0.09)
	Stems	75.5 (3.9)	0.44 (0.01)	0.20 (0.02)	72.6 (5.7)	0.49 (0.08)	0.21 (0.04)
	Whole plant	-	0.68 (0.05)	0.24 (0.04)	-	0.61 (0.04)	0.26 (0.06)
Maize-gr	Grain	50.7 (1.6)	1.51 (0.02)	0.21 (0.02)	50.0 (1.9)	1.53 (0.06)	0.23 (0.05)
Maize-wp	Whole plant	-	1.11 (0.10)	0.16 (0.03)	-	1.06 (0.06)	0.14 (0.01)

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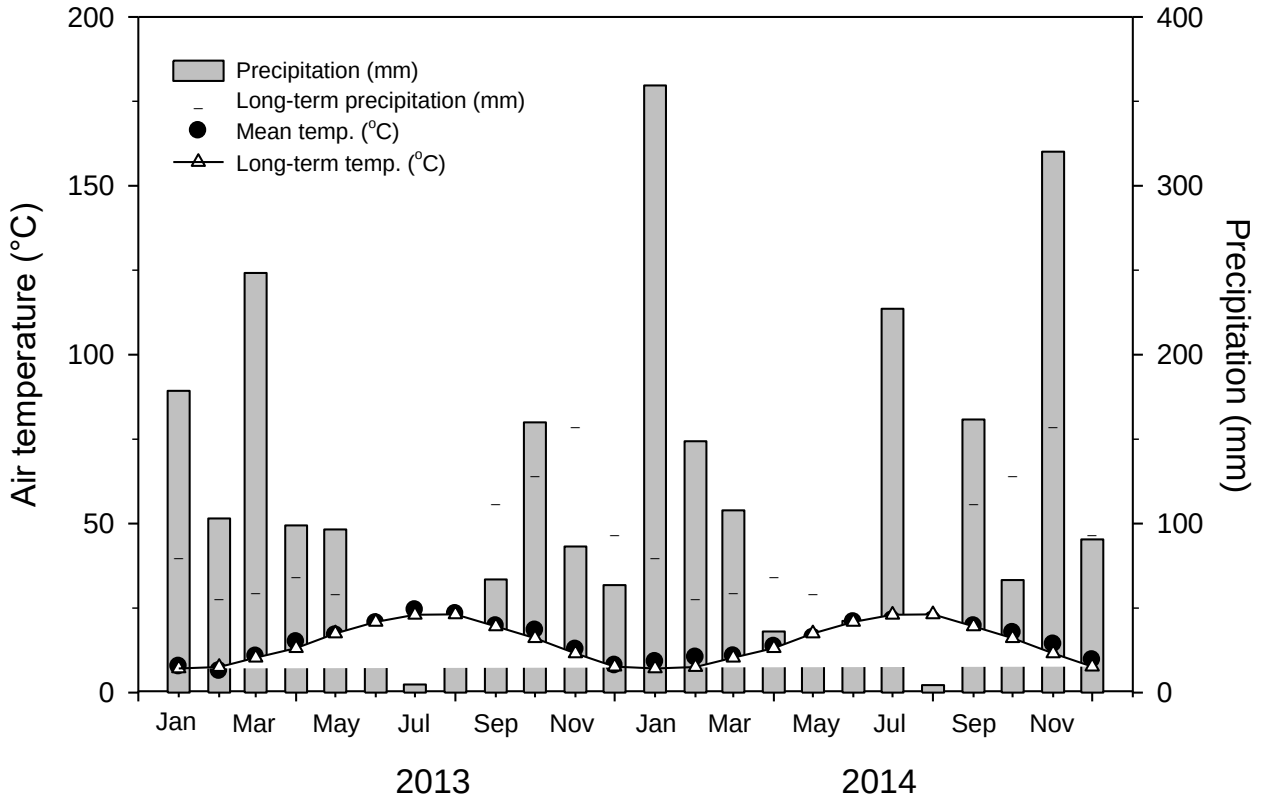


Figure 2: Long-term mean monthly rainfall and temperatures (1989-2014) and monthly rainfall and mean air temperatures in 2013 and 2014 in Vecchiano (Pisa, Italy). The chart is presented as a Bagnouls & Gaussen (1957) diagram to identify dry months, i.e. when the value of rainfall (P, in mm) does not exceed twice the value of mean air temperature (T, in °C) ($P \leq 2T$).

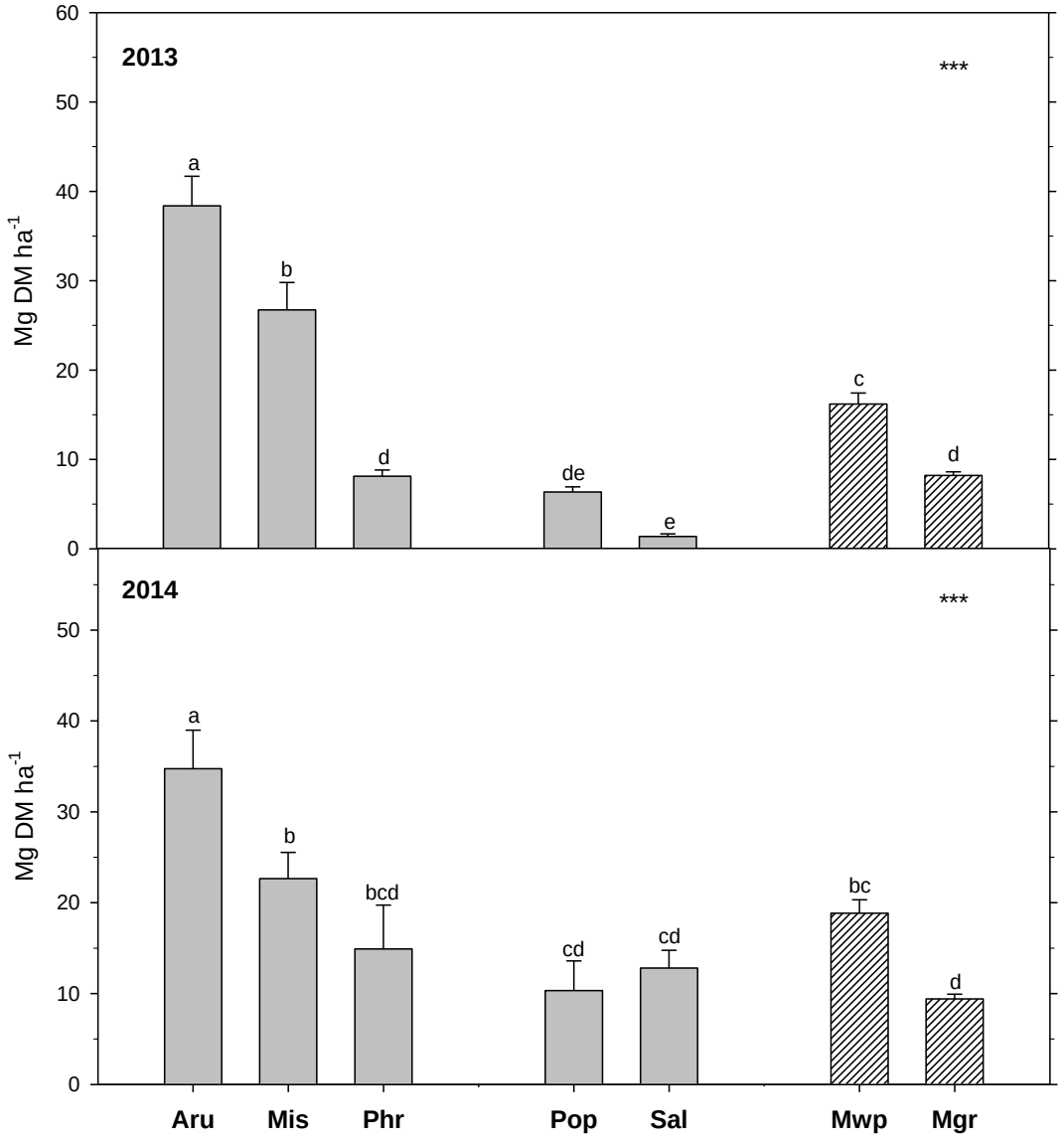


Figure 3: Crop yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus* x *giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Po,: *Poplar* x *canadensis*; Sal, *Salix alba*) and of annual crop (AC: Mwp, maize whole-plant harvest; Mgr, maize grain production). Vertical bars are the mean standard deviations.

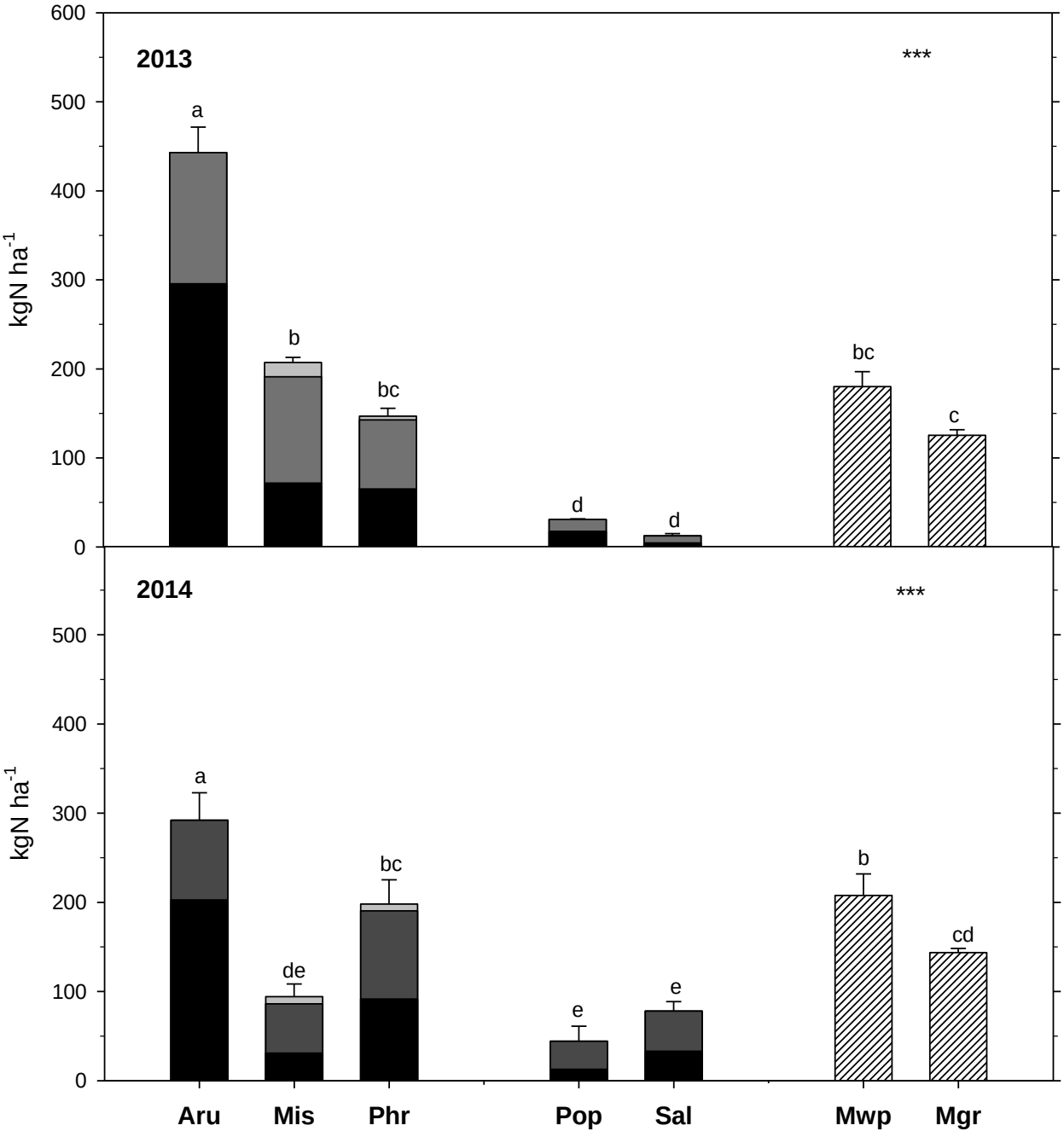


Figure 4: Nitrogen uptake and allocation in different plant parts yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus* x *giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Po,: *Poplar* x *canadensis*; Sal, *Salix alba*) and of annual crop (AC: Mwp, maize whole-plant harvest; Mgr, maize grain production). Black: leaves (PRGs), leaves+branches (SRCs); dark gray: stems (PRGs, SRCs); light gray: panicles (PRGs); diagonal hash marks: maize (AC).

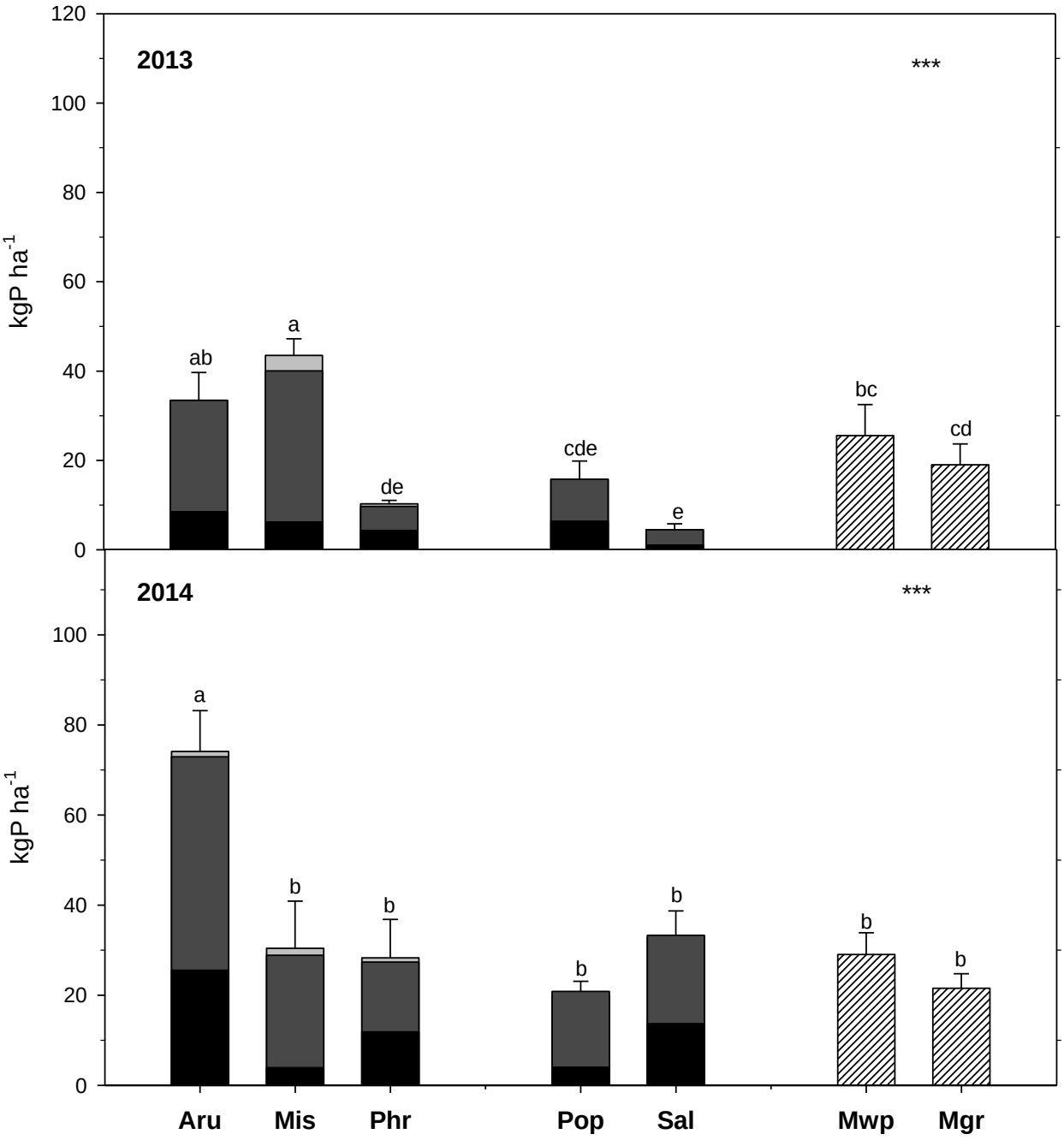


Figure 5: Phosphorus uptake and allocation in different plant parts yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus* x *giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Po,: *Poplar* x *canadensis*; Sal, *Salix alba*) and of annual crop (AC: Mwp, maize whole-plant harvest; Mgr, maize grain production). Black: leaves (PRGs), leaves+branches (SRCs); dark gray: stems (PRGs, SRCs); light gray: panicles (PRGs); diagonal hash marks: maize (AC).

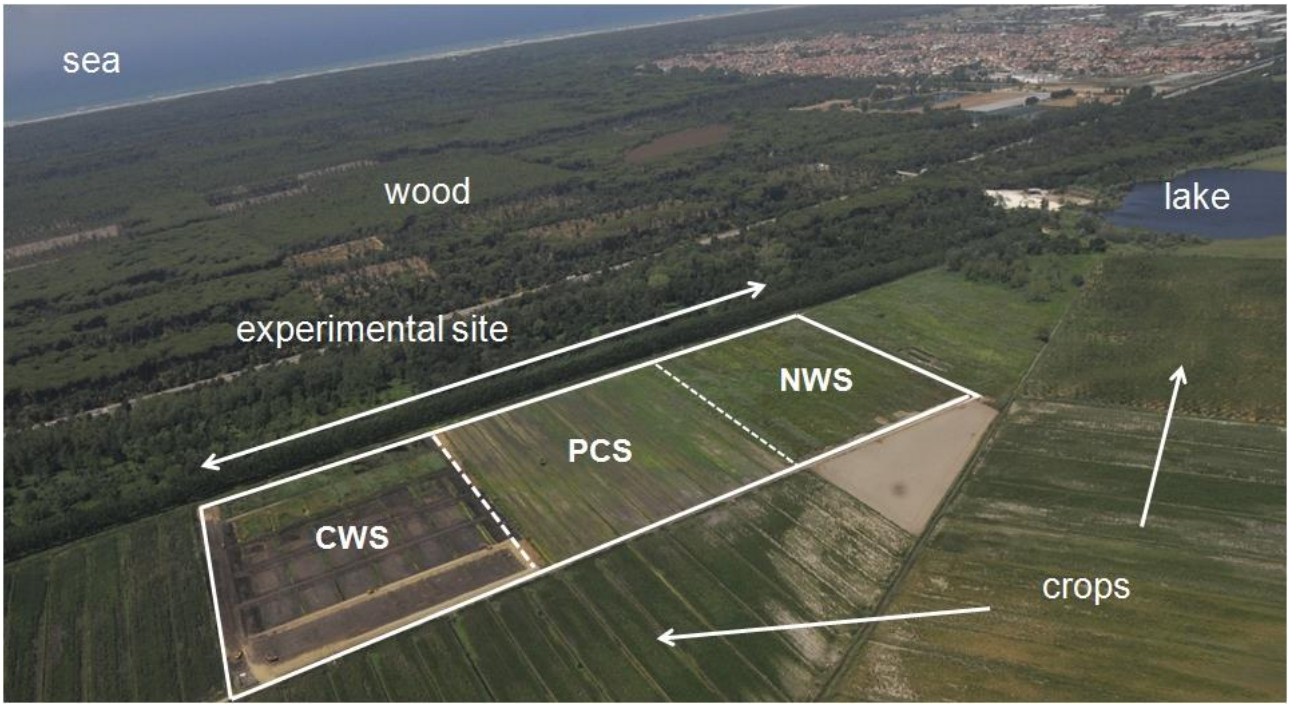


Figure 1. Aerial view of the experimental pilot field represented by three different management systems: constructed wetland system (CWS), paludiculture system (PCS) and natural wetland system (NWS). The conventionally drained area cultivated with annual crops is near the pilot field.