

Growth and nutrient uptake of perennial crops in a paludicultural approach in a drained Mediterranean peatland

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1 **GROWTH AND NUTRIENT UPTAKE OF PERENNIAL CROPS IN A**
2 **PALUDICULTURAL APPROACH IN A DRAINED MEDITERRANEAN**
3 **PEATLAND**

4

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15 **ABSTRACT**

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

25 ‘Oudenberg’), aimed to provide biomass for various bioenergy supply chains. A
26 conventionally cultivated annual crop (maize) was the control. The aim of this study was
27 to compare the sustainability of the proposed paludiculture systems to that of
28 conventional annual crops on the basis of yield and nutrient-removal capability. This 2-
29 year field study evaluated yields, nutrient concentrations and uptake (N and P) of the
30 crops. Over the two years, *A. donax* had the highest mean biomass yield (35 Mg ha⁻¹), N
31 uptake (367 kg ha⁻¹), and P uptake (54 kg ha⁻¹). SRCs had the lowest nutrient uptake in
32 both years. Among grasses, the highest N concentration was recorded in *P. australis*
33 leaves in 2013 (N: 2.41%), while P concentration was greater in *S. alba* branches (P:
34 0.39%). The average above ground biomass of the maize was 17.5 Mg ha⁻¹, while the
35 nutrient uptakes were equal to 194 and 27 kg ha⁻¹ for N and P respectively.
36 Thus, the performances of paludiculture systems were generally encouraging and could
37 represent an important alternative for restoring and managing former drained peatlands
38 in a suitable product chain.

39

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

42

43 **HIGHLIGHTS**

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

48

49 1. INTRODUCTION

50 Peatlands are the most widespread wetland types in the world, representing 50-70% of
51 global wetlands. They cover over four million km², equal to 3% of the land and freshwater
52 surface of the planet (Joosten and Clarke, 2002), and represent not only a major stock of
53 carbon (C) and nitrogen (N), but also a resource of high ecological, historical, recreational
54 and/or agricultural value (Mitch and Gosselink, 2000).

55 In previous centuries, many peatlands were artificially drained as a consequence of
56 increasing land demand for agriculture and forestry (i.e. land-hunger) and the urgent need
57 to improve sanitary conditions (i.e. malaria eradication) for the people living there
58 (Holden et al., 2006). As a consequence, significant changes occurred in physical and
59 chemical properties of the peat (Litaor et al., 2008) such as: i) acceleration of organic-
60 matter oxidation, with a consequent increase in greenhouse gases (GHG) emissions into
61 the atmosphere of up to 25 t CO₂ equivalent ha⁻¹ y⁻¹ (Wichtmann & Wichmann, 2011); ii)
62 increase in NO₃⁻ concentrations in pore water due to higher oxygen availability and the
63 consequent mineralization and nitrification of organic N (Tiemeyer et al. 2007); and iii)
64 mineralization of organic P compounds and increase of sorbed and Fe-bound P pools (Zak
65 et al. 2004). The continual recurrence of these phenomena has negatively affected the
66 land, for example, progressively lowering the soil level (subsidence), increasing nutrient
67 loads delivered to receiving water bodies (eutrophication) and decreasing ecosystem
68 biodiversity and functionality (loss of ecological stability), especially in land-reclamation
69 districts (Schipper & McLeod 2002; Foley et al. 2005; Tiemeyer et al. 2007; Wichtmann
70 & Joosten, 2007; Verhoeven & Setter 2010). For these reasons, rewetting of drained
71 peatlands has been identified since the mid-1990s as an important mitigation strategy to
72 reverse this self-perpetuating process, which is definitely unsustainable (Erwin, 2009).

73 Restoring saturated conditions reduces GHG emissions from the soil, especially CO₂ and
74 NO_x (Joosten & Augustin, 2006). The use of biomass from plants growing on rewetted
75 peatlands (paludiculture) was evaluated to avoid further CO₂ 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 km²) 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-

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

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

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

114 The crops selected for our paludiculture experiment, aiming for high biomass production,
115 rapid development and crop hardiness (Bonari et al. 2004; Angelini et al. 2009; Rowe et
116 al. 2009; Mirza et al. 2010), were perennial rhizomatous grasses (PRG) and short-rotation
117 coppice (SRC) crops. The objective of this study was to evaluate the suitability of the
118 selected crops to paludiculture in a drained Mediterranean peatland, on the basis of their
119 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 m²). Five species were used in the PCS: i) *Populus x canadensis* Moench.
166 var. "Oudenberg" (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 m² (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 m²) 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 H₂SO₄/H₂O₂ (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

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

219

220 **3. RESULTS**

221 **3.1 Plant density and harvestable production**

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

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

227 Among individual species, mean yield over the two years was highest for Aru (36.6 Mg
228 ha⁻¹), followed by Mis (24.7 Mg ha⁻¹) and Maize-wp (17.5 Mg ha⁻¹). Phr (11.5 Mg ha⁻¹)
229 and Pop (8.4 Mg ha⁻¹) yielded lower amounts of biomass, with not statistically different
230 results in 2013 and in 2014. Sal (7.1 Mg ha⁻¹) had the lowest harvestable yields in 2013
231 but had yields similar to those of Phr, Pop, and Maize-gr in 2014.

232 The species in the SRC group showed a different pattern during the experiment. Pop was
233 significantly more productive than Sal in 2013, while Pop and Sal had similar yields in
234 2014. The mean harvestable production of Maize-gr (8.8 Mg ha⁻¹) was similar to that of
235 Pop in 2013 and to those of all SRCs in 2014.

236

237 **3.2 Nutrient concentration**

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

240 affected by the experiment year (Table 3). The highest variability in whole-plant
241 nutrient concentration was found for P: coefficients of variability were high for Aru
242 2013 (22%), Pop 2013 (29%), Mis 2014 (43%), Pop 2014 (30%), and Sal 2014 (23%).
243 Conversely, coefficients of variability of whole-plant N concentrations were higher than
244 20% in only one case (Aru 2014).

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

249

250 **3.3 Plant N uptake**

251 Over the two years, Aru had the highest mean N uptake (367 kg ha^{-1}), followed by Maize-
252 wp (194 kg ha^{-1}), which was not statistically different from Mis and Phr in 2013. N uptake
253 of Mis (mean = 151 kg ha^{-1}) greatly decreased in 2014 (from 207 kg ha^{-1} in 2013 to 94 kg
254 ha^{-1} in 2014) because of a corresponding decrease in N concentration (from 0.78% to
255 0.42%) (Fig.4). Phr had an inverse pattern, in which N uptake increased in 2014 due to
256 greater harvestable production ($+6.80 \text{ Mg ha}^{-1}$). Mean N uptake of Maize-gr (134 kg ha^{-1})
257 ¹) was slightly lower and similar to that of Phr in 2013.. Sal and Pop had the lowest mean
258 N uptake (45 and 38 kg ha^{-1} , respectively) and were significantly different from those of
259 all other species, except for Mis in 2014. Mean N concentrations of PRGs (1.06%) were
260 higher than those of SRCs (0.60%); thus, the pattern of N removal was similar to that of
261 harvestable production, which widens the gap between the two groups of plants. Mean
262 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

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

315 The productivity of woody crops (SRCs) depend greatly on the varieties selected, as well
316 as on soil and environmental conditions in which the species are grown, thus making
317 comparisons among experiments more difficult. At the first cut, a hybrid *Populus* clone,
318 grown in the same coastal plain in Italy at a higher density (10,000 plants ha⁻¹) on a
319 mineral soil managed with a 2-year SRC cycle had a mean yield of about 45 Mg ha⁻¹,
320 which corresponds to a mean of 22.5 Mg ha⁻¹ y⁻¹ (Nassi o di Nasso et al., 2010). The yield
321 recorded in 2014 for Pop (10.3 Mg ha⁻¹), having its roots in the 3rd growing season and
322 one-year-old aboveground organs, was similar to the long-term mean reported by the
323 same study for hybrid poplars (*Populus* sp.) cut every year (9.9 Mg ha⁻¹). Regarding
324 willow (*Salix* sp.), the available data collected in the same area are related to lysimeter
325 experiments on an unfertilized mineral soil, which showed a yield of 6.6 Mg ha⁻¹ in the
326 2nd growing season, which corresponds to 3.3 Mg ha⁻¹ y⁻¹ (Guidi et al., 2008). Typical
327 biomass yields of willow and poplar in European climates range from 3-12 Mg ha⁻¹ y⁻¹
328 (Kauter et al., 2003; Keoleian & Volk, 2005), while maximum yields under optimal
329 conditions can reach 28-30 Mg ha⁻¹ y⁻¹ (Don et al., 2012). However, it should be noted
330 that biomass yields reported in the literature depend greatly on the experimental set-up.
331 Higher yields are generally obtained from experiments with small plots, and therefore
332 significant edge effects, while lower yields are reported for large plots and open-field
333 cultivation (Kauter et al., 2003). Overall, under our experimental conditions, interesting

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

336 Maize showed no relevant differences in yield between the two years, as similar and
337 remarkable harvestable yields were observed (Maize-gr: 8.2 and 9.4 Mg ha⁻¹ in 2013 and
338 2014, respectively; Maize-wp: 16.2 and 18.8 Mg ha⁻¹ in 2013 and 2014, respectively).

339 This pattern indicated that maize, as observed by Silvestri et al. (2012), can achieve high
340 productivity under the hydraulic conditions present in the reclamation district,
341 characterized by a shallower water depth compared to other areas in the region.

342 Comparison with yields obtained in the same area shows that productivity of the rainfed
343 maize was strongly affected by water availability. Maize subject to a shallow water table
344 had whole-plant yields in line with our results (8-16 Mg ha⁻¹) (Bellocchi et al., 2002),
345 while a deeper water table led to lower yields (6-10 Mg ha⁻¹) (Mazzoncini et al., 2011).

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

356 Conversely, willow and poplar showed increasing yields (62% and 830%, respectively),
357 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 CO₂, CH₄ and N₂O 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 km²), 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

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

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

439

440 **5. CONCLUSIONS**

441 Restoration of degraded peatland represents an important issue at both local and global
442 scales. Agricultural use of peatland based on traditional drainage and cropping systems is
443 becoming progressively less adequate, and new management strategies should be defined.

444 Paludiculture may represent an option that preserves agricultural use of land and reverses
445 peatland degradation, which is particularly intense in Mediterranean conditions.

446 The findings of the present study demonstrate the agronomic suitability of the crops tested
447 and showed some of the environmental benefits achievable, although a need exists for a
448 longer experimental period to reach steady-state conditions. Results revealed the great
449 influence of species on biomass yields and nutrient uptake, which represent important
450 points for paludiculture system design, along with other aspects that should be
451 investigated (e.g. harvest time, water table level control, lifespan). The choice of an
452 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

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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

464 **FIGURE CAPTIONS**

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

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

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

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

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

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

501 **TABLE CAPTIONS**

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

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

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

510

511

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710

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 ₂ O ₅	120 kg ha ⁻¹ of P ₂ O ₅
potassium fertilization***	60 kg ha ⁻¹ of K ₂ O	90 kg ha ⁻¹ of K ₂ O
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

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719 Table 3: Mean plant-tissue nutrient contents of plant species in the 2013 and 2014 growing
720 seasons. Aru: *Arundo donax*; Mis: *Miscanthus x giganteus*; Phr: *Phragmites australis*; Pop:
721 *Populus x canadensis*; Sal: *Salix alba*; Maize-wp, maize, whole plant; Maize-gr, maize, grain
722 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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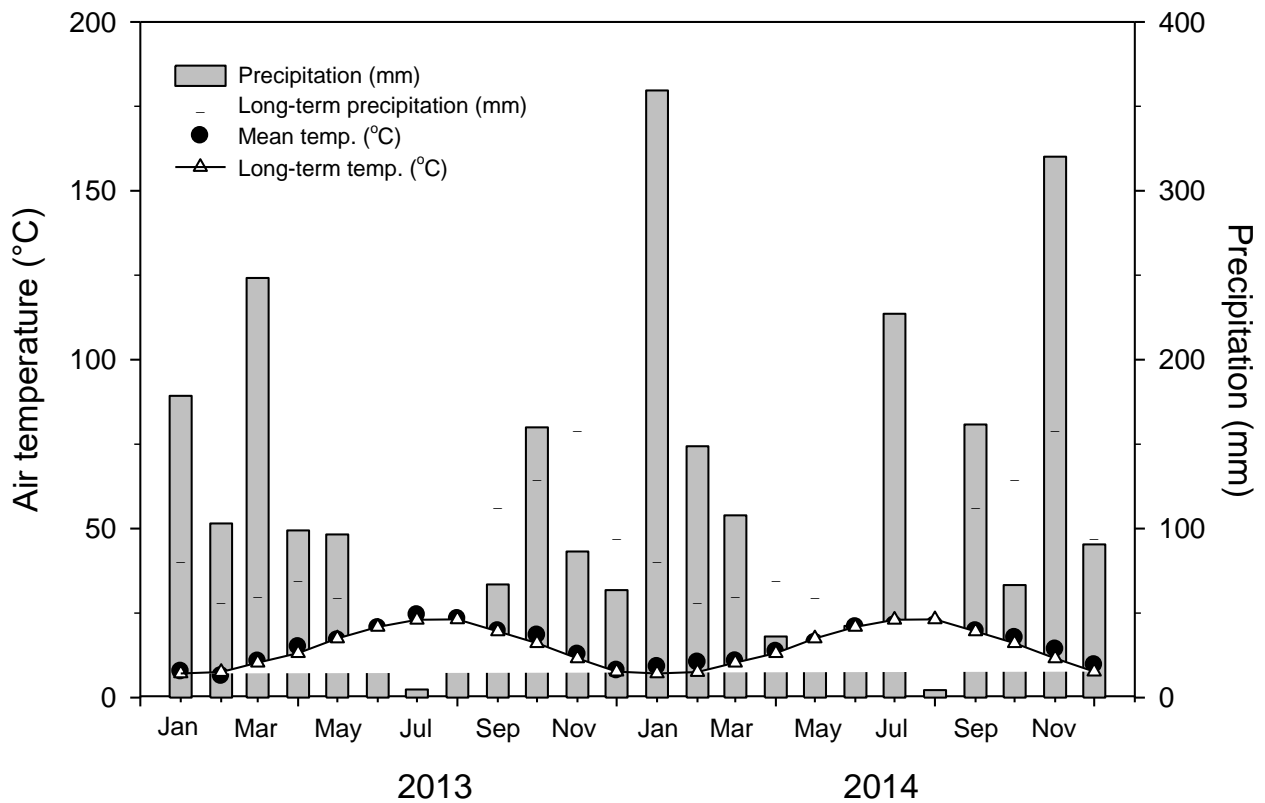


Figure 2: Long-term mean monthly rainfall and temperatures (1989-2014) and monthly rainfall and mean air temperatures in 2013 and 2014 in Vecchiano (Pisa, Italy). The chart is presented as a Bagnouls & Gausson (1957) diagram to identify dry months, i.e. when the value of rainfall (P , in mm) does not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

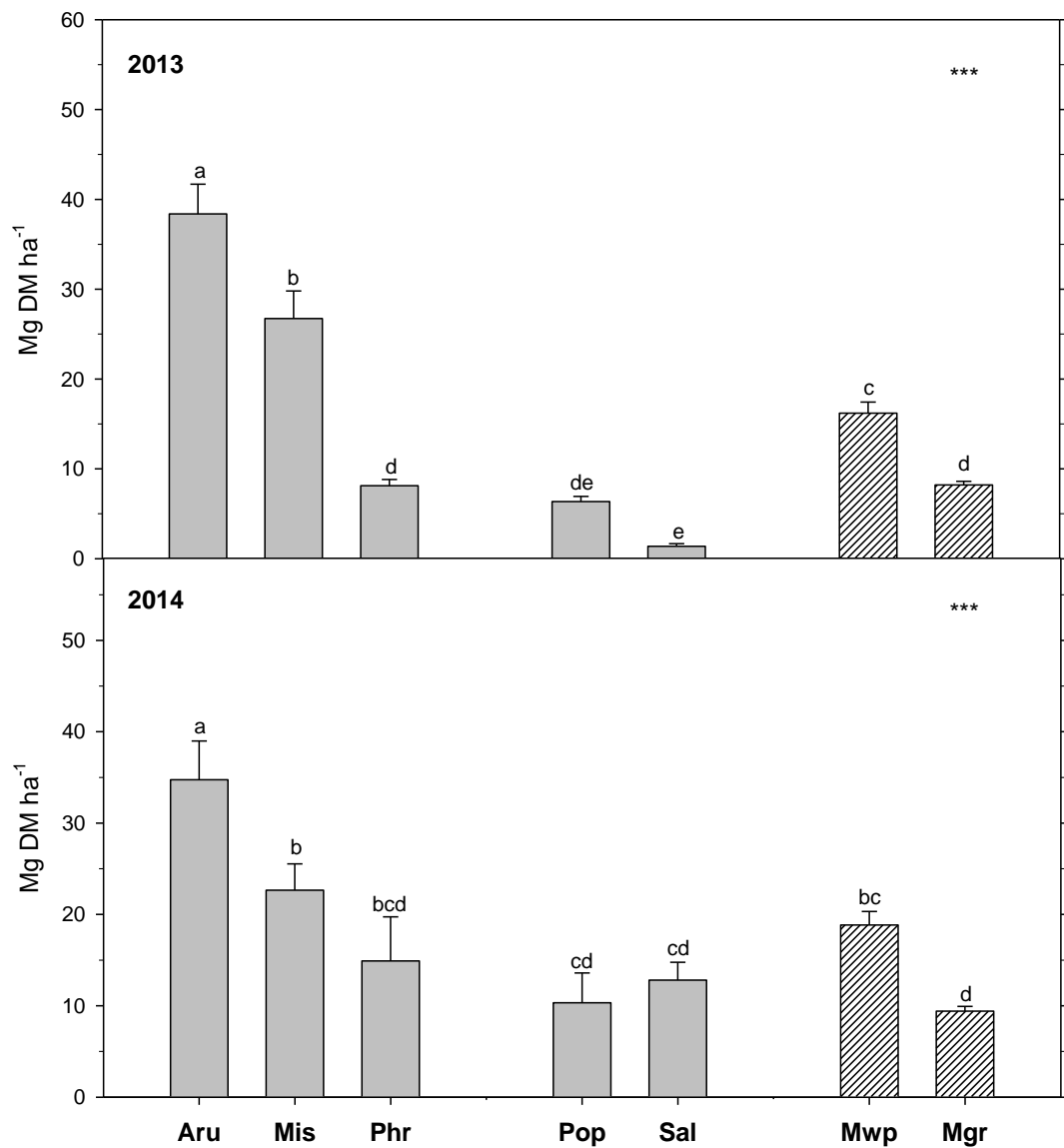


Figure 3: Crop yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus x giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Po., *Poplar x canadensis*; Sal, *Salix alba*) and of annual crop (AC: Mwp, maize whole-plant harvest; Mgr, maize grain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

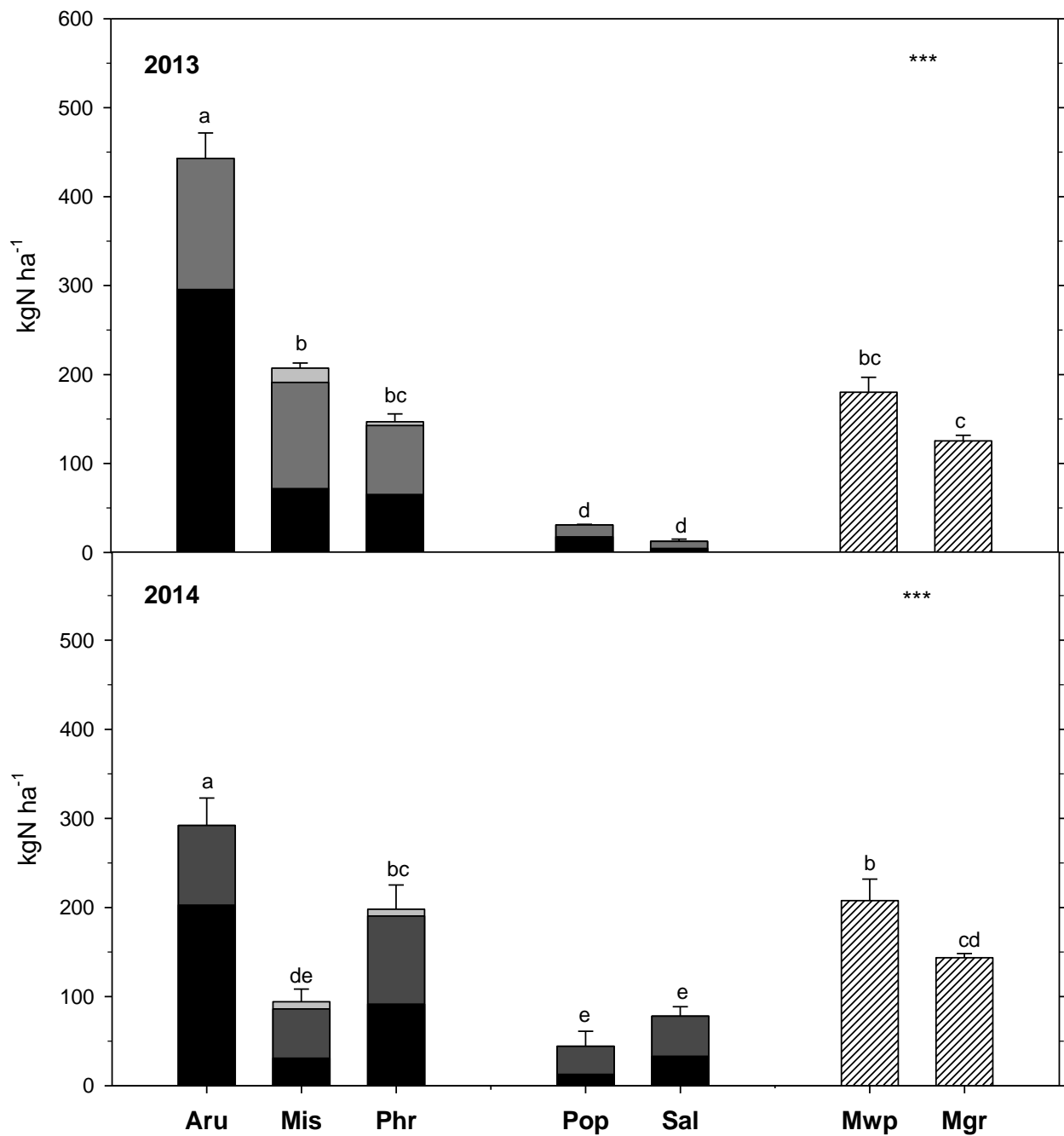


Figure 4: Nitrogen uptake and allocation in different plant parts yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus x giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Po., *Poplar x canadensis*; Sal, *Salix alba*) and of annual crop (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).

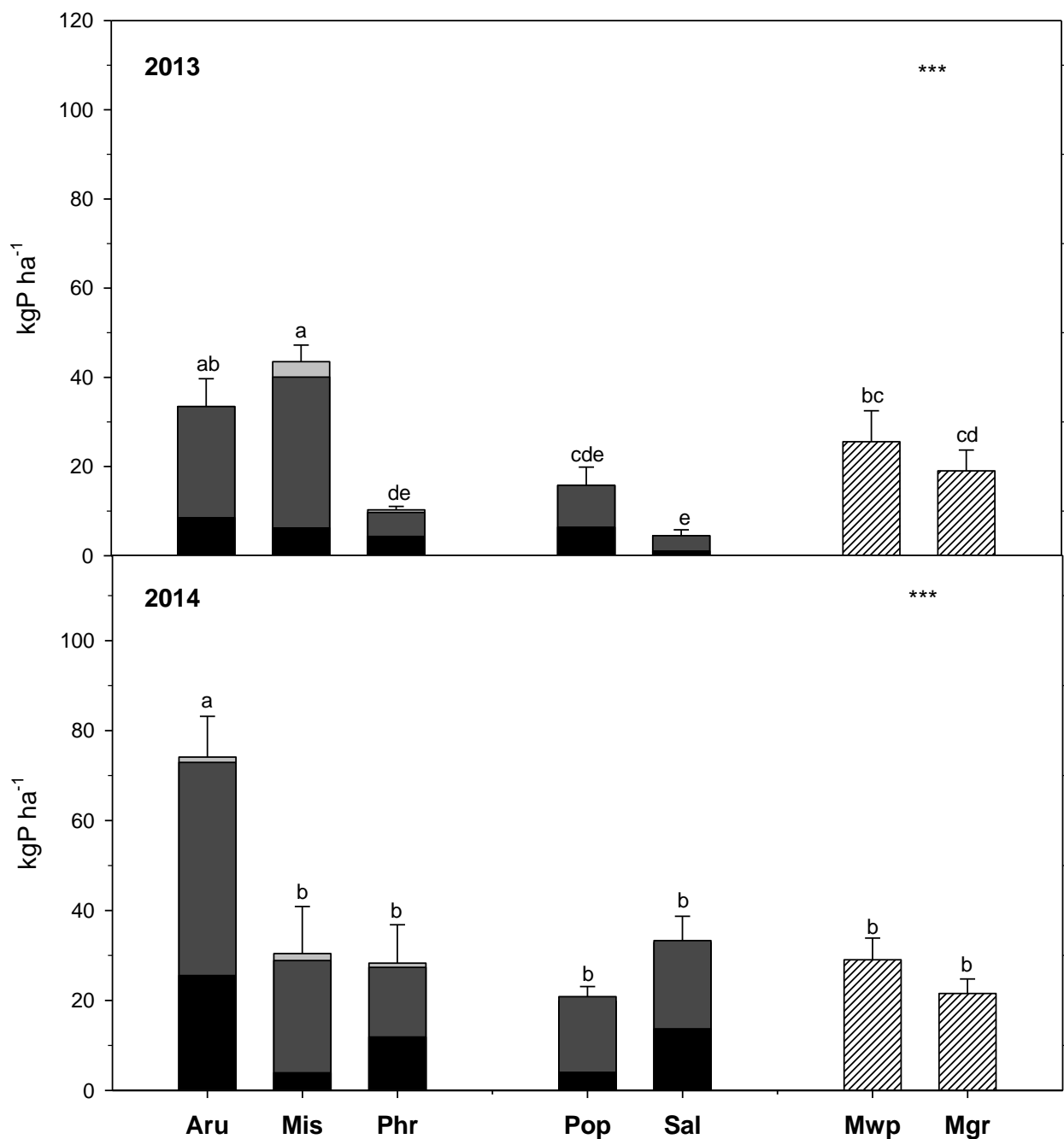


Figure 5: Phosphorus uptake and allocation in different plant parts yields of perennial rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus x giganteus*; Phr, *Phragmites australis*), of short rotation coppice species (SRCs: Po., *Poplar x canadensis*; Sal, *Salix alba*) and of annual crop (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).

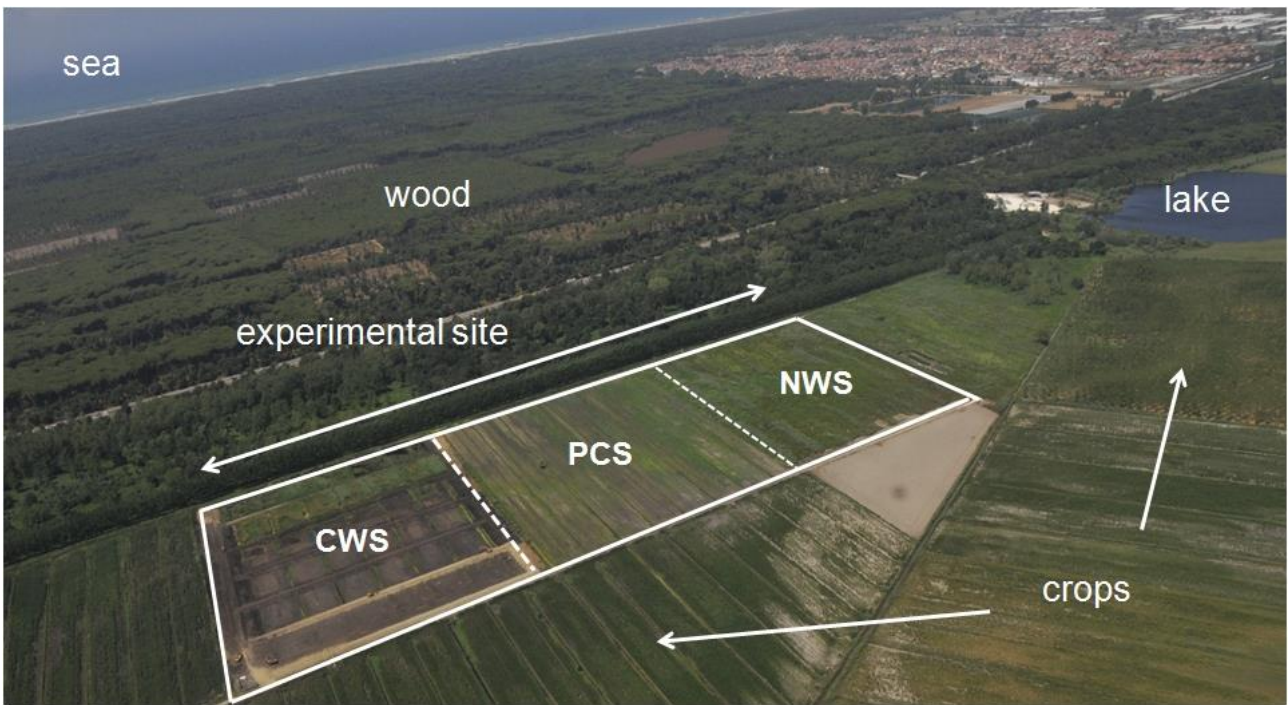


Figure 1. Aerial view of the experimental pilot field represented by three different management systems: constructed wetland system (CWS), paludiculture system (PCS) and natural wetland system (NWS). The conventionally drained area cultivated with annual crops is near the pilot field.