

1 **GROWTH AND NUTRIENT UPTAKE OF PERENNIAL CROPS IN A**
2 **PALUDICULTURAL APPROACH TO RESTORE A DRAINED**
3 **MEDITERRANEAN 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 phosphorus enrichment of surface waters. An
22 experimental trial was established in spring 2012 with three perennial rhizomatous
23 grasses (PRG) (*Phragmites australis*, *Miscanthus* × *giganteus*, *Arundo donax*) and two
24 woody species managed as short-rotation coppice (SRC) (*Salix alba* 'Dimitrios',

25 *Populus ×canadensis* ‘Oudenberg’) to provide biomass for various bioenergy supply
26 chains. A conventionally cultivated annual crop (maize) was the control. The aim of this
27 study was 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 performances of paludiculture systems were generally encouraging and
35 could represent an important alternative for restoring and managing former drained
36 peatlands in a suitable product chain.

37

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

40

41 **HIGHLIGHTS**

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

46

47 **1. INTRODUCTION**

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

53 In previous centuries, many peatlands were artificially drained as a consequence of
54 increasing land demand for agriculture and forestry (i.e. land-hunger) and the urgent
55 need to improve sanitary conditions (i.e. malaria eradication) for the people living there
56 (Holden et al., 2006). As a consequence, significant changes occurred in physical and
57 chemical properties of the peat (Litoar et al., 2008) that led to i) acceleration of organic-
58 matter oxidation, with a consequent increase in greenhouse gas (GHG) emissions into
59 the atmosphere of up to 25 t CO₂ equivalent ha⁻¹ y⁻¹ (Wichtmann & Wichmann, 2011);
60 ii) increase in NO₃⁻ concentrations in pore water due to higher oxygen availability and
61 the consequent mineralization and nitrification of organic N (Tiemeyer et al. 2007); and
62 iii) mineralization of organic P compounds that does not necessarily involve an increase
63 in soluble reactive phosphorus concentration (Zak et al. 2004). The continual recurrence
64 of these phenomena has negatively affected the land, for example, progressively
65 lowering the soil level (subsidence), increasing nutrient loads delivered to receiving
66 water bodies (eutrophication) and decreasing ecosystem biodiversity and functionality
67 (loss of ecological stability), especially in land-reclamation districts (Schipper
68 & McLeod 2002; Foley et al. 2005; Tiemeyer et al. 2007; Wichtmann & Joosten, 2007;
69 Verhoeven & Setter 2010). For these reasons, rewetting of drained peatlands has been
70 identified since the mid-1990s as an important mitigation strategy to reverse this self-

71 perpetuating process, which is definitely unsustainable (Erwin, 2009). Restoring
72 saturated conditions reduces GHG emissions from the soil, especially CO₂ and NO_x
73 (Joosten & Augustin, 2006). The use of biomass from plants growing on rewetted
74 peatlands (paludiculture) was evaluated to avoid further CO₂ emissions by replacing raw
75 fossil materials and fuels.

76 Paludiculture is defined as the agricultural use of wet and rewetted peatlands to produce
77 biomass for bioenergy (e.g. direct combustion, biogas, biofuels) or other purposes (e.g.
78 feed; fiber; raw materials for industrial biochemistry, pharmaceuticals and cosmetics),
79 which slows subsidence and nutrient release from the peat soil (reduction of
80 mineralization rate, plant uptake and harvest) and improves ecosystem services (e.g.
81 habitat restoration, aquifer recharge, nutrient cycling) (Wichtmann & Tanneberger
82 2011; Joosten et al. 2012; Abel et al. 2013; Gunther et al. 2014).

83 In Mediterranean areas, especially in Italy, this approach is quite new because of a
84 historical tradition that always considered the drainage of wetlands (land reclamation) a
85 necessary condition for the development and well-being of human communities.
86 Although peatlands cover less than 1% (about 300 km²) of Italy's national territory, the
87 vast majority was drained for agricultural purposes in the 1920s-1930s.

88 Our case study is located in a reclamation district on the coastal plain of west-central
89 Italy and is characterized by large-scale, intensive agriculture and the presence of a
90 vulnerable receiving water body, Lake Massaciuccoli. Since the 1930s, a complex
91 network of artificial drains and pumping stations has been used to drain the superficial
92 aquifer and excess rainfall, thus ensuring a water table depth suitable for cultivation
93 (Ciccolini et al. 2013). The lake and surrounding marches are Wetlands of International
94 Importance according to the Ramsar Convention since 2014, but their status is seriously

95 harmed by severe eutrophication. Furthermore, the traditional agricultural use of the
96 land-reclamation district is seriously compromised by increasing difficulties in
97 maintaining the unsaturated zone for crop growth (Zuccarini et al., 2011; Pistocchi et
98 al., 2012). For these reasons, it is necessary to identify suitable alternative management
99 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
104 those of annual crops, making their cultivation possible in fields not easily accessible
105 due to 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
109 reducing eutrophication risk for the receiving water body. Furthermore, high yields may
110 increase farmers' incomes and possibly ensure economic sustainability of the system
111 (Tzanakakis et al., 2009). Increasing fixation of carbon dioxide in organic compounds
112 decreases GHG emissions into the atmosphere and provides more matter to renovate the
113 peat stock, which limits subsidence (Joosten et al., 2014).

114 The crops selected for our paludiculture experiment, aiming for high biomass
115 production, rapid development and crop hardiness (Bonari et al. 2004; Angelini et al.
116 2009; Rowe et al. 2009; Mirza et al. 2010), were perennial rhizomatous grasses (PRG)
117 and short-rotation coppice (SRC) crops. The objective of this study was to compare the

118 suitability of the paludiculture option to that of the usual farming system based on
119 annual crop cultivation in terms of biomass yields and nutrient uptake.

120

121 **2. MATERIALS AND METHODS**

122 **2.1 Study site**

123 The research was conducted over two years (2013-2014) on part of a larger
124 phytotreatment system located in Vecchiano, about 10 km from Pisa, Italy (43° 49'
125 59.5" N; 10° 19' 50.7" E) in the Migliarino-San Rossore-Massaciuccoli Natural Park
126 (<http://www.parcosanrossore.it>).

127 This 15 ha experimental area was used to compare the efficiency of three different
128 systems in treating the eutrophic drainage water coming from a sub-watershed in the
129 reclamation district. These systems have different types of water management (water
130 level and path) and plant management (species, cultivation and harvesting) (Ciccolini et
131 al. 2013). The systems tested were a constructed wetland system (CWS), a natural
132 wetland system (NWS), and a paludiculture system (PCS) (Fig. 1). The last of these was
133 based on growing different non-food crops and harvesting their biomass periodically to
134 ensure nutrient removal from the fields. The system was not dammed and was crossed
135 by a dense network of small channels (about 8 m apart) that supplied both drainage (in
136 autumn and winter) and irrigation (in spring and summer) for the crops through lateral
137 infiltration. The soils of the PCS (Table 1) were classified as Histosol according to the
138 USDA system (Soil Survey Staff, 1975) and as Rheic Histosol according to the FAO
139 system (IUSS, 2006). They are representative of the soils of the land-reclamation
140 district, which are also defined as peat and peaty soils (Pellegrino et al., 2014). The
141 main difference between the PCS and the surrounding areas concerns the water table

142 level. The water table in the watershed is adjusted for farming, with noticeable
143 fluctuations during the year (-0.10 to -0.60 m), while in the rewetted PCS the water
144 level is stable, ranging from 0.0 to -0.15 cm. The climate is Mediterranean (Csa)
145 according to the Köppen–Geiger climate classification map (Kottek et al., 2006).
146 Summers are dry and hot, rainfall is mainly concentrated in autumn and spring (mean
147 annual rainfall = 945 mm) and mean monthly air temperature at 2 m ranges from 7°C in
148 February to 30°C in August (mean = 14.8 °C). Mean monthly temperatures and rainfall
149 for 2013-2014 and over the long term (1989-2014) were recorded at a weather station
150 located in the Massaciucoli basin (Fig. 2).

151

152 **2.2 Trial set up**

153 Five species were used in the PCS: i) *Populus x canadensis* Moench. var. "Oudenberg"
154 (Pop) and *Salix alba* L. var. "Dimitrios" (Sal), belonging to the SRC group; and ii)
155 *Arundo donax* L. (Aru), *Miscanthus × giganteus* Greef et Deuter (Mis), and *Phragmites*
156 *australis* L. (Phr), belonging to the PRG group. Maize (*Zea mays* L.) was the annual
157 crop (AC) used as the control; it is the most widespread crop in this area, cultivated for
158 grain production in continuous cropping or two-year rotations with winter wheat or
159 sunflower (Silvestri et al., 2012). Maize was cultivated in the fields surrounding the
160 phytotreatment system under rainfed conditions, since the shallow water table supplied
161 sufficient water to the crop. Two maize cultivation purposes were considered: grain
162 production (Mgr) and whole-plant harvest (Mwp).

163 Before planting, the land was uncultivated and dominated by spontaneous vegetation
164 (Pellegrino et al., 2014). Crops were established in spring 2012; however, the first year

165 of cultivation (2012) was not assessed because the phytotreatment system did not
166 operate until January 2013.

167 Tillage was performed in autumn 2011 by plowing, followed by rotary harrowing
168 immediately before planting. Mis and Phr were planted at a density of two rhizomes per
169 m² (1.0 x 0.5 m spacing, 20,000 rhizomes per ha), while Aru was planted using pre-
170 rooted plants at the same density as that of the other PRGs. The SRCs Sal and Pop were
171 established using one-year-old dormant cuttings (2.0 x 0.75 m spacing, 6,600 plants per
172 ha).

173 No fertilizers or pesticides were applied to the crops. Weed control was mechanical for
174 SRCs (until machines could gain access to the inter-rows) and manual for PRGs.
175 Irrigation was provided during the first growing season, from establishment to October
176 2012, to supply water until the phytotreatment system began operating. The harvest was
177 annual for PRGs and bi-annual for SRCs. Maize agronomic management (Table 2)
178 reflected farmers' usual practices in the area.

179

180 **2.3 Data collection and processing**

181 Aboveground biomass yield of each crop was measured in both years of the experiment
182 (2013 and 2014). The PRGs and AC were harvested in late September-early October,
183 while SRCs were collected in December. For PRGs and AC, samples were obtained by
184 pooling three sub-samples (2 m²) for each field replicate. For each sample, the number
185 of plants and the biomass fresh weight were recorded. For PRGs and AC, 10 stems and
186 4 plants, respectively, were partitioned into leaves, stems and panicles or grains, and the
187 fresh weight of separated parts was determined. SRC plant density was measured over a
188 30 m linear distance. Next, three representative transects of six consecutive plants were

189 harvested for each replicate. Leaves and branches were separated from the stem, and the
190 fresh weights were recorded for the overall sampled biomass and the different plant
191 parts. Yield per ha was calculated as a function of observed plant density. Afterwards,
192 all subsamples were dried at 60°C until a constant weight to determine the dry matter
193 contents of all plant components and the dry matter yields of the crops (Mg ha^{-1}). The
194 dried samples were ground to 1 mm and used for tissue-nutrient analyses following
195 digestion of 200 mg of plant material by $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ (Bremner 1965). N concentration
196 was determined with the Kjeldahl method, while P concentration was determined with
197 the molybdenum blue method using a Perkin Elmer Lambda 25 spectrophotometer
198 (Giannini et al., 2014). Nutrient uptake (kg ha^{-1}) for each plant part was calculated as
199 the product of its nutrient concentration and dry biomass weight. The overall nutrient
200 uptake of each crop was calculated as the sum of the uptake of each part.

201 Data were analyzed according to a completely randomized design, with the crop as
202 treatment and three replicates represented by the field sections between two adjacent
203 channels ($8 \text{ m} \times 300 \text{ m} = 2400 \text{ m}^2$). When significant differences were observed, means
204 of different plant species were compared using Tukey's honest significant difference
205 (HSD) post-hoc test at the 0.05 p-level. All statistical analyses were performed with R
206 statistical software (version 3.1.1, R Foundation for Statistical Computing).

207

208 **3. RESULTS**

209 **3.1 Plant density and harvestable production**

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

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

216 Among individual species, mean yield over the two years was highest for Aru (36.6 Mg
217 ha⁻¹), followed by Mis (24.7 Mg ha⁻¹) and Mwp (17.5 Mg ha⁻¹). Phr (11.5 Mg ha⁻¹) and
218 Pop (8.4 Mg ha⁻¹) yielded lower amounts of biomass, with statistically equivalent
219 results in 2013 and in 2014. Sal (7.1 Mg ha⁻¹) had the lowest harvestable yields in 2013
220 but had yields equivalent to those of Phr, Pop, and Mgr in 2014.

221 The species in the SRC group showed a different pattern during the experiment. Pop
222 was significantly more productive than Sal in 2013, while Pop and Sal had equivalent
223 yields in 2014. The mean harvestable production of Mgr (8.8 Mg ha⁻¹) was similar to
224 that of Pop in 2013 and to those of all SRCs in 2014.

225

226 **3.2 Nutrient concentration**

227 Nutrient concentrations in the tissues of different species varied greatly depending on
228 the plant part analyzed (leaves, stems, branches, panicles, grain, etc.) and were also
229 affected by the experiment year (Table 3). The highest variability in whole-plant
230 nutrient concentration was found for P: coefficients of variability were high for Aru
231 2013 (22%), Pop 2013 (29%), Mis 2014 (43%), Pop 2014 (30%), and Sal 2014 (23%).
232 Conversely, coefficients of variability of whole-plant N concentrations were higher than
233 20% in only one case (Aru 2014).

234 The highest N concentrations (calculated as weighted means) of harvestable plant parts
235 were for Phr (1.85% and 1.33% in 2013 and 2014, respectively) and Mgr (1.51% and
236 1.53% in 2013 and 2014, respectively), while Sal had the highest P concentrations

237 (0.32% and 0.26% in 2013 and 2014, respectively). High concentrations were measured
238 for N in Mwp and Aru (0.86-1.15%) and for P in Pop and Mgr (0.20-0.24%).
239 Mis had low concentrations of both N (0.78% and 0.42% in 2013 and 2014,
240 respectively) and P (0.16% and 0.14% in 2013 and 2014, respectively). Among plant
241 parts, the highest nutrient concentrations were found for N in the leaves of Aru (2.43%
242 and 2.16% in 2013 and 2014, respectively) and for P in the branches of Sal (0.31% and
243 0.39% in 2013 and 2014, respectively).

244

245 **3.3 Plant N uptake**

246 Over the two years, Aru had the highest mean N uptake (367 kg ha^{-1}), followed by Mwp
247 (194 kg ha^{-1}), which was statistically equivalent to Mis and Phr in 2013 but only to Phr
248 in 2014. N uptake of Mis (mean = 151 kg ha^{-1}) greatly decreased in 2014 (from 207 kg
249 ha^{-1} in 2013 to 94 kg ha^{-1} in 2014) because of a corresponding decrease in N
250 concentration (from 0.78% to 0.42%). Phr had an inverse pattern, in which N uptake
251 increased in 2014 due to greater harvestable production ($+6.80 \text{ Mg ha}^{-1}$). Mean N
252 uptake of Mgr (134 kg ha^{-1}) was slightly lower and equivalent to that of Phr in 2013 and
253 higher than that of Mis in 2014. Sal and Pop had the lowest mean N uptake (45 and 38
254 kg ha^{-1} , respectively) and were significantly different from those of all other species,
255 except for Mis in 2014, which was equivalent to that of Sal.

256 Mean N concentrations of PRGs (1.06%) were higher than those of SRCs (0.60%); thus,
257 the pattern of N removal was similar to that of harvestable production (Fig. 4), which
258 widens the gap between the two groups of plants. Mean maize N concentrations were
259 the highest (Mgr = 1.52% and Mwp = 1.09%), which made its N uptake more similar to
260 those of PRGs and consequently increased differences with performances of the SRCs.

261

262 **3.4 Plant P uptake**

263 Over the two years, Aru had the highest mean P uptake (54 kg ha^{-1}); however, Mis P
264 uptake was higher in 2013 ($43 \text{ vs } 33 \text{ kg ha}^{-1}$) because of the low P concentration in Aru
265 biomass (0.09%). Mean P uptake by Mwp and Mgr was even lower ($27 \text{ and } 20 \text{ kg ha}^{-1}$,
266 respectively), while those for the other species (Sal, Phr, and Pop) were similar to each
267 other ($18\text{-}20 \text{ kg ha}^{-1}$).

268 The pattern of P uptake was different for the two years. In 2013, Mis and Aru had
269 significantly higher P uptake than the other species, whose P uptake values were
270 equivalent in pairs (Mwp = Mgr, Mgr = Pop, Pop = Phr, Phr = Sal). In 2014, Aru and
271 Sal had the highest and lowest P uptake, respectively, while those of the other species
272 were statistically equivalent (Mis = Phr = Sal = Mwp = Mgr).

273 Mean P concentration was higher in SRCs (0.27%) than in PRGs (0.15%), leading to a
274 smaller relative difference in P uptake (Fig. 5) compared to N uptake. Mean P
275 concentration of Mgr (0.22%) lay between those of PRGs and SRCs, while that of Mwp
276 (0.15%) was the same as those of PRGs. This led to a general flattening of the values of
277 P uptake, particularly in 2013 (max = $43 \text{ and } 5 \text{ kg ha}^{-1}$ for Mis and Sal,
278 respectively), but which was also noticeable in 2014 (max = $74 \text{ and } 20 \text{ kg ha}^{-1}$ for
279 Aru and Pop, respectively).

280

281

282 **4. DISCUSSION**

283 To assess the suitability of different species, it is important to evaluate their adaptability
284 and productive capacity under paludicultural conditions. Adaptability is inferred by

285 comparing yields in the paludiculture system with those obtained under similar
286 conditions (i.e. climate, growing season) in unsaturated soils (intraspecific assessment),
287 while productive capacity is evaluated by comparing harvestable productions of the
288 species under paludicultural conditions to those of traditional annual crops (interspecific
289 assessment).

290 All the PRGs tested showed good adaptability to tested conditions. Mean yields of Aru
291 and Mis over the two years, corresponding to their 2nd and 3rd growing seasons, were
292 36.5 and 25.0 Mg ha⁻¹, respectively, which are similar to those obtained at the same
293 crop age in a mineral soil with a lower water table level in the same area (the coastal
294 plain of western-central Italy) (Angelini et al., 2005, 2009; Roncucci et al., 2014).
295 Harvestable yields of Aru were also similar to those obtained in southern Italy by Borin
296 et al. (2013) in mineral soils irrigated with nutrient-rich water (37 and 29 Mg ha⁻¹ in the
297 2nd and the 3rd growing seasons, respectively). In contrast, Mis yields observed in the
298 same area by Angelini et al. (2009) were slightly higher (48 and 29 Mg ha⁻¹ in the 2nd
299 and the 3rd growing seasons, respectively). Therefore, cultivation of Aru and Mis in a
300 paludicultural cropping system did not seem to penalize their potential productivity, and
301 they adapted well to rewetting conditions in the two years considered.

302 The production of Phr is more difficult to evaluate because of the lack of experiments in
303 the same area. However, despite lower yields than the other PRGs, Phr was considered
304 adaptable by several authors, since it typically grows under wetland conditions (Graneli,
305 1984; Egloner, 2009; Wichtmann and Couwnberg, 2013). Comparison with other Italian
306 experiments highlights that the mean yield of Phr in this study (11.5 Mg ha⁻¹) was
307 higher than those reported by Molari et al. (2014) on four sites, in which biomass
308 production ranged from 2.3-8.5 Mg ha⁻¹ on mineral soils irrigated with nutrient-rich

309 waters simulating agricultural-drainage effluents. Köbbing et al. (2013) reported yields
310 ranging from 5-20 Mg ha⁻¹ in northern Europe and China. Hence, in this study Phr had
311 biomass yields in line with those reported in the literature for this species.

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

333 Maize had no relevant differences in yield between the two years, as similar and
334 remarkable harvestable yields were observed (Mgr: 8.2 and 9.4 Mg ha⁻¹ in 2013 and
335 2014, respectively; Mwp: 16.2 and 18.8 Mg ha⁻¹ in 2013 and 2014, respectively). This
336 pattern indicated that maize can achieve high productivity under these hydraulic
337 conditions (shallow water table) as observed by Silvestri et al. (2012). Comparison with
338 yields obtained in the same area shows that productivity of the rainfed maize was
339 strongly affected by water availability. Maize subject to a shallow water table had
340 whole-plant yields in line with our results (8-16 Mg ha⁻¹) (Bellocchi et al., 2002), while
341 a deeper water table led to lower yields (6-10 Mg ha⁻¹) (Mazzoncini et al., 2011).

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

352 Conversely, willow and poplar showed increasing yields (62% and 830%, respectively),
353 probably in relation to the progressive development of their root systems, with a
354 consequent increase in production capability (Wilkinson, 1999). Phr also had higher
355 harvestable biomass in 2014 than in 2013 (+84%), which could be explained by the
356 higher shoot density in the second year (data not shown). Maize (Mgr and Mwp)

357 showed no noticeable difference in yield between the two years (+14% for 2014), in
358 relation to its annual crop cycle and to the agronomic practices provided each year
359 (Table 2).

360 Due to the harvestable yield trends that might be observed in future years, it is still not
361 possible to identify the most suitable crop for this paludicultural system. Therefore, the
362 experimental period should be extended for several years to evaluate crop behavior at a
363 steady-state, after crops have spent a few years under saturated soil conditions and have
364 reached maturity. Moreover, the sustainability of the paludiculture option also depends
365 on environmental aspects and the profitability of these peatland management systems.

366 In particular, with the aim to decrease nutrient concentrations in water to be treated,
367 assessing crop nutrient-uptake rates is crucial to maximize the benefits of plant use. In
368 our study, mean amounts of nutrients removed were high for Aru (367 kg N ha⁻¹ and 54
369 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
370 and 19 kg P ha⁻¹). These quantities are of the same order of magnitude as the nutrient
371 loads carried to the PCS by the water (up to 350 kg N and 25 kg P ha⁻¹ y⁻¹ if the water-
372 treatment system operates 365 days per year), indicating that plant uptake can remove a
373 significant portion of the nutrients introduced into the system. In Aru, about 68% of N
374 and 30% of P was contained in the leaves, thus highlighting their relevant role at harvest
375 in nutrient removal by this species. Conversely, concentrations in leaves were lower in
376 the other PRGs, mostly due to a lower leaf-mass ratio (Mis) and to higher nutrient
377 concentrations in the stems (Phr). This can have practical implications in timing crop
378 harvests. The species in which foliar mass plays a relevant role in uptake and early
379 harvests are tolerated (i.e. Aru) (Dragoni et al., 2015) could be harvested before
380 senescence, thus preventing leaf loss and increasing nutrient removal. However, Mis's

381 lower contribution of leaves to overall uptake and lower tolerance of early cuts (Strullu
382 et al., 2013) means that it could benefit from delayed harvests. In turn, harvest timing
383 has potential effects on nutrient relocation to belowground organs and thus on the
384 lifespan of PRG plantations (Lewandowski et al., 2003).

385 Regarding Pop and Sal, the amounts of nutrients removed are lower than those of other
386 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
387 leaves represent a large portion of the plants that cannot be collected, since most fall to
388 the ground during conventional harvest times (autumn-winter), and early harvests are
389 not advisable, since they can greatly reduce the lifespan of the crop stands (Sennerby-
390 Forsse et al., 1992). Therefore, most leaves are excluded from nutrient removal,
391 although they can temporarily immobilize non-negligible amounts of nutrients and help
392 build peat stocks. As reported by Tzanakakis et al. (2009), the quantity of leaves
393 produced by *Populus* sp. in the 2nd and 3rd growing seasons was about 8-9% of total
394 accumulated biomass, which equals a temporary uptake of 17-20% and 11-15% of the
395 total N and P, respectively, taken up by the plant. An analogous contribution to C and
396 nutrient immobilization can be envisaged for maize stover left in the field when only
397 grain is collected (Mgr). Moreover, maize had relatively high nutrient uptake,
398 particularly when the entire plant was harvested (Mwp: 194 kg N ha⁻¹ and 27 kg P ha⁻¹;
399 Mgr: 134 kg N ha⁻¹ and 20 kg P ha⁻¹). In this case, it should be considered that maize
400 cultivation also involves fertilizer inputs, making the balance between removals and
401 contributions of nutrients almost equal.

402 Ceasing agricultural activities will not significantly reduce the annual rate of soil
403 organic matter mineralization (Pellegrino et al., 2014), and only prolonging saturated
404 conditions in the soil can slow down the chemical and physical subsidence (Wichtmann

405 and Wichmann, 2011). Achieving these conditions represents the main objective in
406 managing the degraded peatland, and paludiculture can combine two difficult issues: i)
407 maintaining agricultural use of a reclamation district and ii) affordably restoring
408 degraded peatland.

409 Economic sustainability of paludiculture systems seems an essential prerequisite for its
410 spread in the territory. At the productivity levels observed in this study, the expected
411 revenues of the AC would be reached by SRCs and PRGs at selling prices from equal to
412 3-4 times lower than that of maize. Therefore the profitability of PCS seems related to
413 the limiting of cultivation cost, in particular those of two specific work-chains: planting
414 and harvesting.

415

416 **5. CONCLUSIONS**

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

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

429 adaptive approach (paludiculture) in peatland management instead of a transformative
430 approach (land reclamation) would require an important change in attitude in land
431 planning, agronomy and farming.

432

433 **ACKNOWLEDGMENTS**

434 This work was supported by the “Consorzio di Bonifica Versilia-Massaciuccoli” and
435 funded by the “Regione Toscana”. The authors wish to thank the technical staff of the
436 Consorzio di Bonifica Versilia-Massaciuccoli for managing the experimental site and
437 harvesting biomass from the entire experimental pilot field, and Fabio Taccini and
438 Sergio Cattani for their help sampling and milling biomass.

439

440

441 **FIGURE CAPTIONS**

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

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

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

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

463 **Fig. 5:** Phosphorus uptake and allocation in different plant parts yields of perennial
464 rhizomatous grasses (PRGs: Aru, *Arundo donax*; Mis, *Miscanthus x giganteus*; Phr,

465 *Phragmites australis*), of short rotation coppice species (SRCs: Po,: *Populus x*
466 *canadensis*; Sal, *Salix alba*) and of annual crop (AC: Mwp, maize whole-plant harvest;
467 Mgr, maize grain production). Black: leaves (PRGs), leaves+branches (SRCs); dark
468 gray: stems (PRGs, SRCs); light gray: panicles (PRGs); diagonal hash marks: maize
469 (AC).

470

471 **TABLE CAPTIONS**

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

474 **Table 2:** Main maize cultivation practices for grain production (Mgr) and whole-plant
475 harvest (Mwp).

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

480

481

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Figure1
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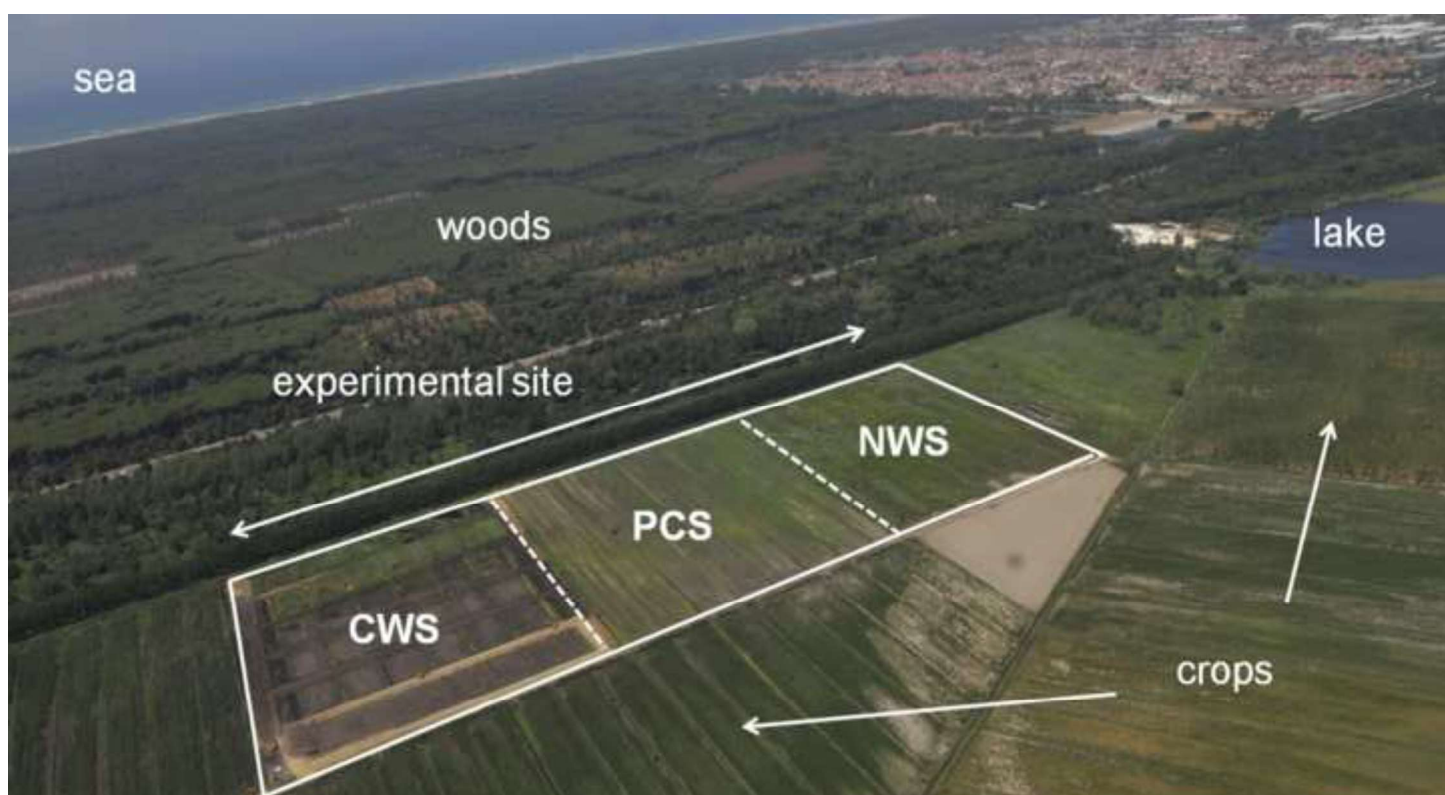


Figure2

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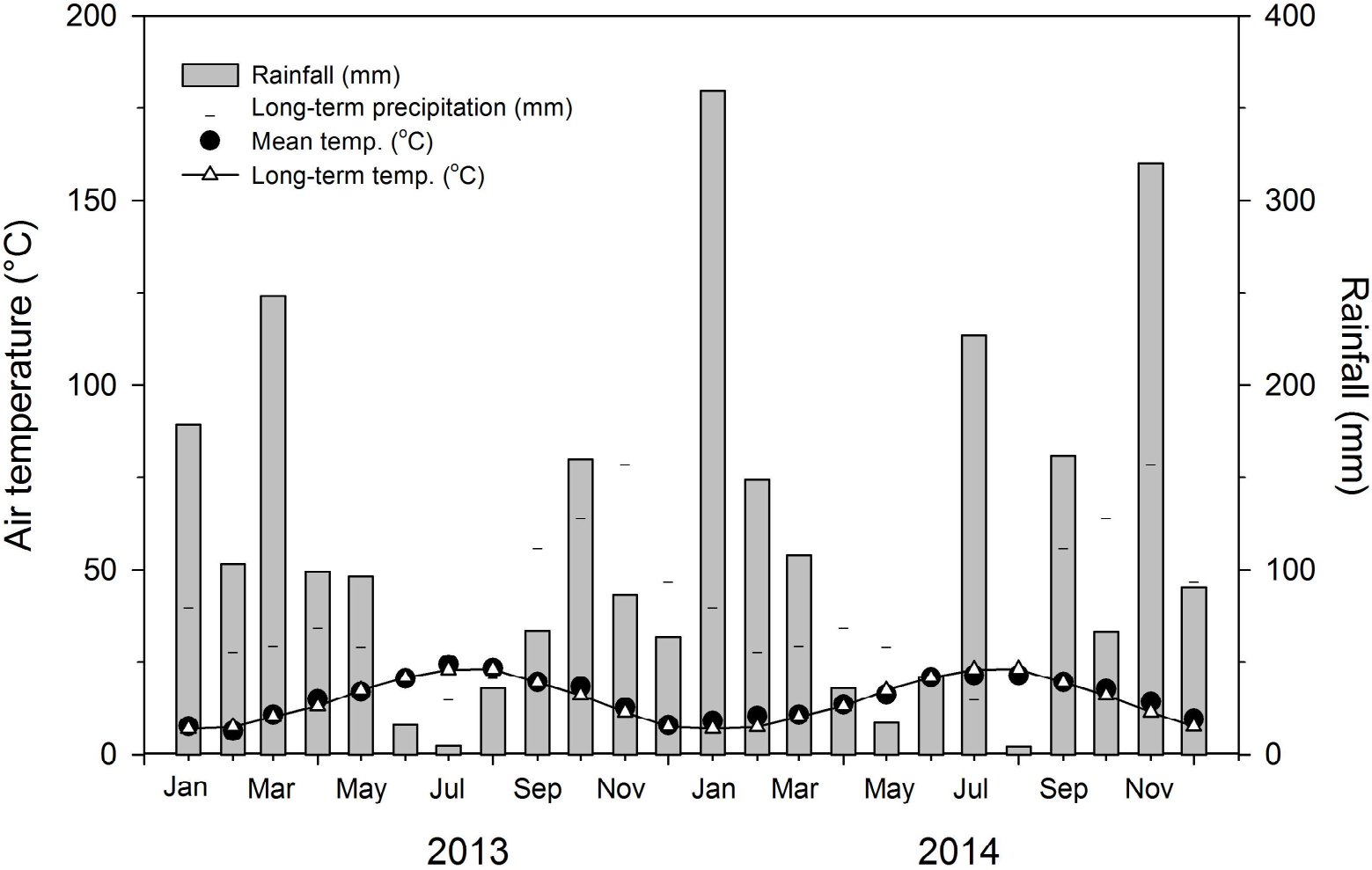


Figure3

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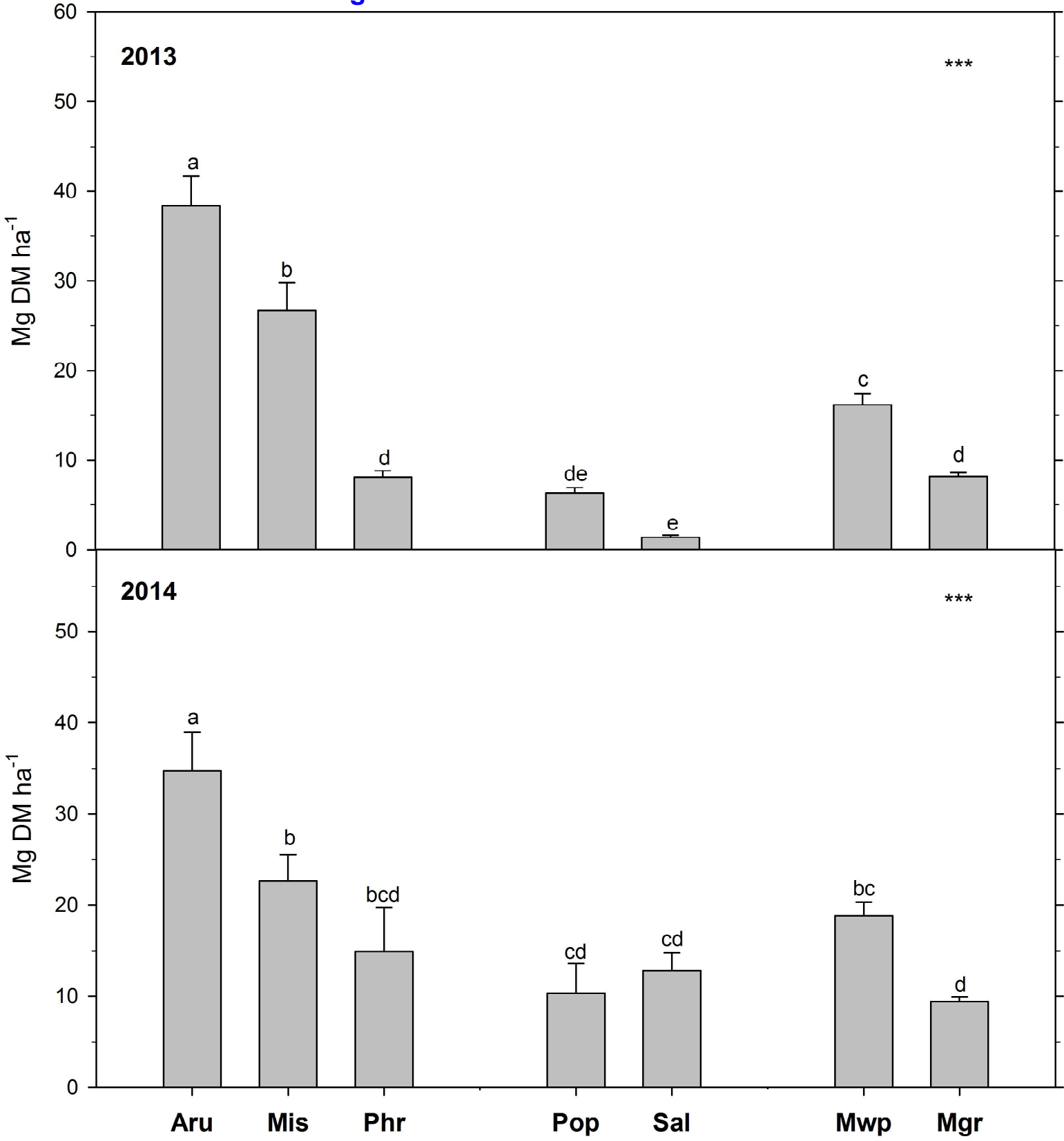


Figure4

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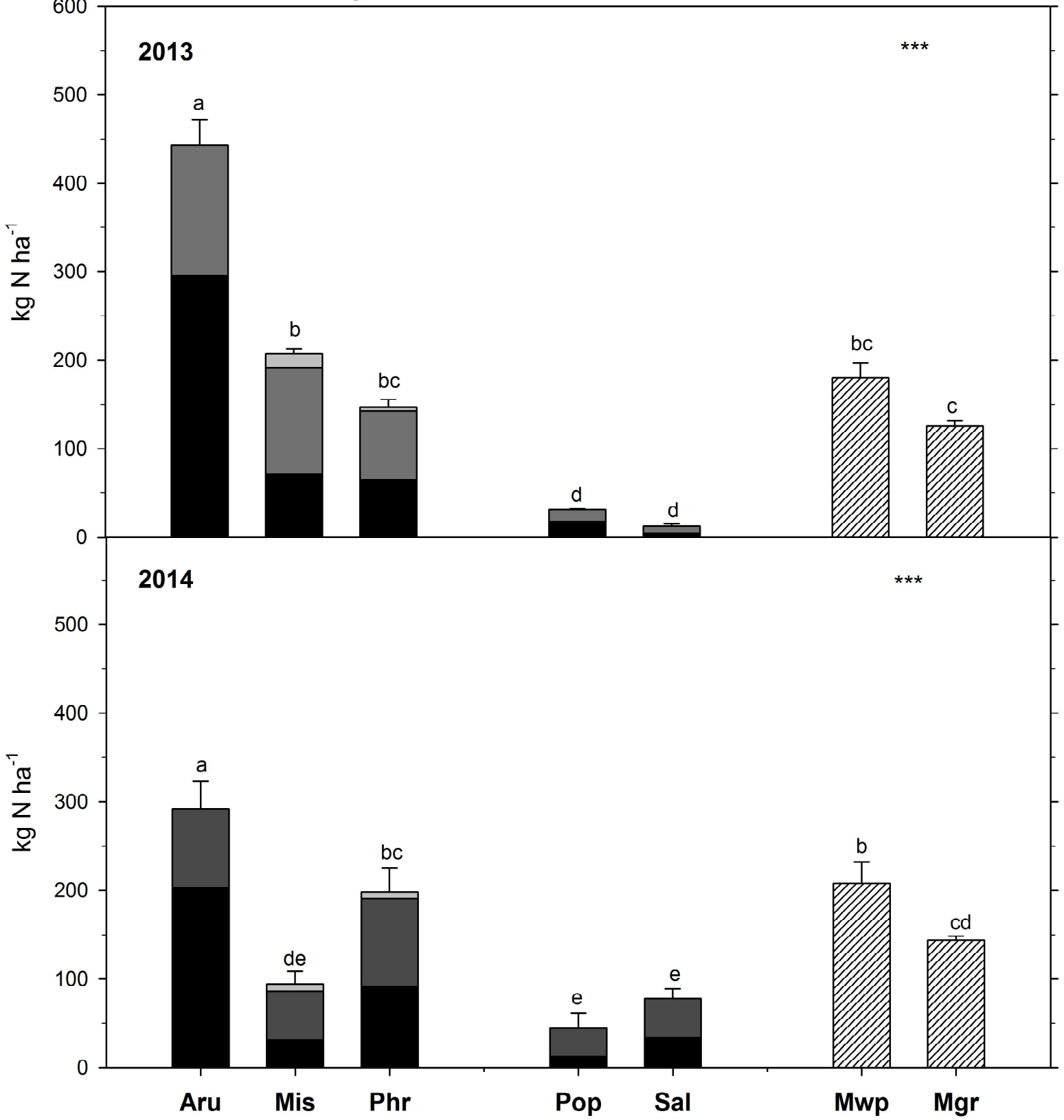


Figure5

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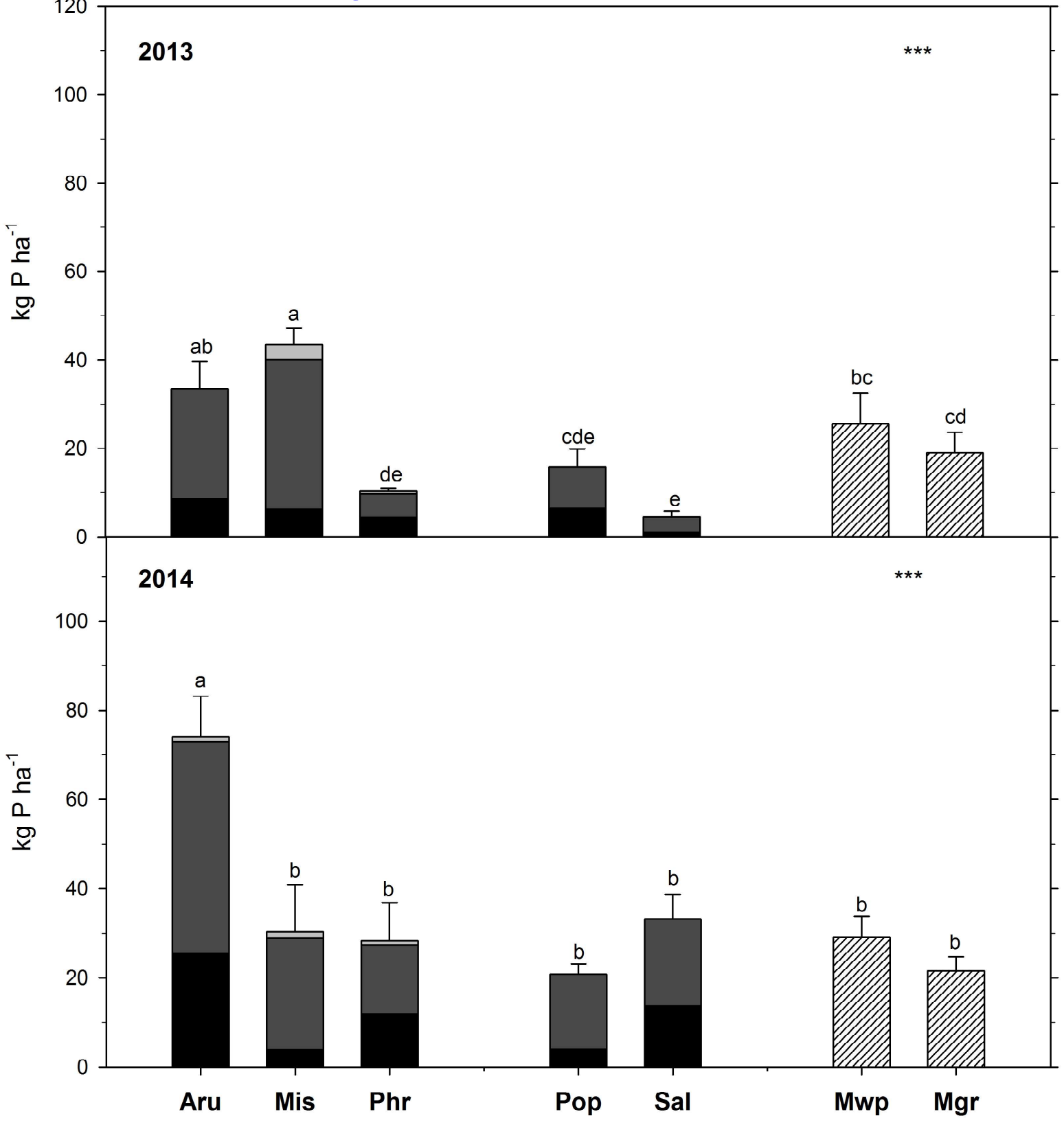


Table 1. Physical and chemical characteristics of paludiculture system soils (0-30 cm depth). *atomic absorption; **extractable with ammonium oxalate.

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

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

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: *Poplar x canadensis*; Sal: *Salix alba*; Mwp, maize, whole plant; Mgr, 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)
Mgr	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)
Mwp	Whole plant	-	1.11 (0.10)	0.16 (0.03)	-	1.06 (0.06)	0.14 (0.01)