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Mycorrhizal status of Cyperaceae from New Caledonian ultramafic soils: effects of phosphorus availability on arbuscular mycorrhizal colonization of *Costularia comosa* under field conditions

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33	Keywords separated by ' - '	Ultramafic soils - Arbuscular mycorrhiza - Phosphorus - Cyperaceae - <i>Costularia</i> - Field experiment
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Mycorrhizal status of Cyperaceae from New Caledonian ultramafic soils: effects of phosphorus availability on arbuscular mycorrhizal colonization of *Costularia comosa* under field conditions

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Abstract Plants from the Cyperaceae family (sedges), usually considered as non-mycorrhizal, constitute almost exclusively the herbaceous stratum of the ultramafic maquis in New Caledonia. These plants are pioneers and are important for the ecological restoration of mined areas. *Costularia comosa*, one of the most common sedges in this environment, was grown under field conditions on ultramafic soil, fertilized or not with phosphate and/or nitrogen. Results showed that the addition of phosphate to the soil induced a clear increase in mycorrhizal colonization of *C. comosa* and an increase in arbuscule abundance, reflecting the establishment of a functional mycorrhizal symbiosis. Significant positive correlations were found among mycorrhizal parameters and plant or soil phosphorus concentrations. Nitrogen fertilization did not affect mycorrhizal colonization of *C. comosa*. The improvement in mycorrhizal colonization by phosphate fertilization did not influence significantly nickel concentrations in the roots and shoots of plants. This study demonstrated that phosphate fertilization of ultramafic soil improved mycorrhizal colonization of *C. comosa*, with formation of a functional symbiosis under field conditions.

Keywords Ultramafic soils · Arbuscular mycorrhiza · Phosphorus · Cyperaceae · *Costularia* · Field experiment

Introduction

Ultramafic ecosystems (serpentine) occupy one third of the main island in the tropical South Pacific island group of New Caledonia. Ultramafic soils are characterized by very low concentrations of phosphate (P), nitrogen (N), and Ca and high levels of Ni, Co, Mn, and Cr (Brooks 1987). About 89 % of the vascular plants in the ultramafic maquis are endemic to New Caledonia (Jaffré et al. 2004). Among them, Cyperaceae species (sedges) are pioneers and constitute almost exclusively the herbaceous stratum (Jaffré 1992). These plants are therefore potentially important for the revegetation of mined areas. *Costularia* is the most widespread genus of the Cyperaceae family in ultramafic maquis in New Caledonia, with 12 species being endemic (Jaffré et al. 2004). *Costularia comosa* (C.B. Clarke) Kük is frequently found in all the ultramafic regions of New Caledonia and is particularly recommended for ecological restoration (Wulf et al. 2010).

Cyperaceae are generally considered as non-mycorrhizal or very weakly mycorrhizal (Miller et al. 1999; Wang and Qiu 2006; Brundrett 2009). However, some publications have reported the presence of arbuscular mycorrhizal (AM) associations within Cyperaceae (Amir et al. 1997; Muthukumar et al. 2004; Gai et al. 2006; Perrier et al. 2006). Lagrange et al. (2011) demonstrated that even a low level of mycorrhizal colonization is functional in *C. comosa* plants grown in ultramafic soil under greenhouse conditions. These authors hypothesized that in natural conditions, mycorrhizal colonization of Cyperaceae could be inhibited by phosphorus deficiency, as suggested by Lambers et al. (2009) for extremely poor soils.

Previously reported field experiments on ultramafic soils have concerned the effects of fertilization on growth and plant species dynamics (Ferreira and Wormell 1971; Carter

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- 72 et al. 1988; Chiarucci and Maccherini 2007), without con- 119
 73 sidering mycorrhizal symbiosis. The aim of the present 120
 74 study was to evaluate the effects of mineral phosphate 121
 75 and/or nitrogen fertilization on the mycorrhizal status of *C.* 122
 76 *comosa*, under field conditions on ultramafic soils. 123
- 77 **Materials and methods** 124
- 78 **Experimental site description** 125
- 79 New Caledonia is located approximately 1,500 km east of 126
 80 Australia and 1,800 km north of New Zealand in the South 127
 81 Pacific. The climate is subtropical to tropical, with a rainy 128
 82 season from December to May and a dry season from 129
 83 September to November that can vary in duration and se- 130
 84 verity. Mean monthly temperature in the capital, Noumea, is 131
 85 highest in February with 26.2 °C and lowest in August with 132
 86 19.9 °C. The experimental site is located in an ultramafic 133
 87 ligno-herbaceous maquis in the plum area (22°16'59"S, 134
 88 166°39'12"E). The soil of this site is a highly weathered 135
 89 geric ferralsol (Becquer et al. 2001) with the following 136
 90 physicochemical characteristics: 4.56 % organic carbon; 137
 91 0.22 % total N; C/N 20.0; pH 5.34 (H₂O); 192.4 mg kg⁻¹ 138
 92 total P; 3 mg kg⁻¹ available P (Olsen); 0.09 % Ca; 0.72 % 139
 93 Mg; 93.6 mg kg⁻¹ Na; 682.7 mg kg⁻¹ K; 37.8 % Fe; 1.43 % 140
 94 Mn; 0.54 % Ni; 0.12 % Co; 3.67 % Cr; 170 mg kg⁻¹ Mg 141 Q3
 95 (dimethylenetriaminopentaacetic acid (DTPA)); 1,116 mg 142
 96 kg⁻¹ Mn (DTPA); 106 mg kg⁻¹ Fe (DTPA); 116 mg kg⁻¹ Ni 143
 97 (DTPA); 79 mg kg⁻¹ Co (DTPA); and 0.6 mg kg⁻¹ Cr (DTPA). 144
- 98 **Field experiment** 145
- 99 The experiment aimed at assessing the effect of P and/or N 146
 100 fertilization on the mycorrhizal status and growth of *C.* 147
 101 *comosa* in ultramafic soil conditions formed part of a project 148
 102 of field production of Cyperaceae seeds for ecological res- 149 Q4
 103 toration conducted by the firm SIRAS Pacific and the ex- 150
 104 perimental plan involved other aspects not presented here 151
 105 (plant flowering, seed production, etc.). The natural vegeta- 152
 106 tion was removed and the soil homogenized using agricul- 153
 107 tural equipment; any invading plants were regularly 154
 108 removed. A completely randomized experimental block de- 155
 109 sign typically used for agronomic assays was chosen to take 156
 110 into account the heterogeneity of the experimental field 157
 111 (Dagnélie 2003). The experimental field was divided into 158
 112 five blocks: one block represented a homogeneous and flat 159
 113 planting area bounded by 2.5 m wide strips of natural 160
 114 vegetation (maquis). These strips were left in their natural 161
 115 state to minimize erosion and runoff, which could induce 162
 116 transfer of mineral inputs and particles from one block to 163
 117 another. The field sloped gently (5 %); the five flat blocks 164
 118 being perpendicular to the slope. Each block was divided 165
- into four plots separated by a 1-m-wide path and corre- 119
 sponding to the four combinations of N and P fertilization 120
 treatments, arranged randomly. Each plot was an experimen- 121
 tal unit of 12 m² containing 24 plants of *C. comosa*, corre- 122
 sponding to one treatment. The plants were distributed in 123
 two planting lines, 50 cm apart. Overall, for each treatment, 124
 5 plots of 24 plants were assessed. 125
- C. comosa* plants were produced in a nursery and were 126
 aged 12 months when planted into the field in April 2006. 127
C. comosa seeds were obtained from an ultramafic maquis 128
 near lake Yaté (south New Caledonia). Plants were firstly 129
 grown 16 months in a nursery (the plants grow very slowly) 130
 at 23–28 °C, in small pots (170 cm³) containing a mix of 131
 coco fiber (30 % v/v), commercial compost (10 %), and old 132
 topsoil (60 %), before planting them in the experimental 133
 field. The level of mycorrhizal colonization of the plants 134
 before transplanting was estimated to be less than 0.5 %. 135
- The four fertilization treatments were: N–P– (control with- 136
 out fertilization), N–P+, N+P–, and N+P+. The nitrogen fer- 137
 tilizer used was “medium Azolon 38 N,” a urea– 138
 formaldehyde-based slow-release fertilizer (12 months under 139
 the influence of temperature and humidity) containing 38 % of 140
 total N (2 % of N as urea and 36 % as methylene urea). 141 Q3
 Nitrogen (N+ treatment) was added at 500 kg of N per ha. 142
 Phosphorus (P+ treatment) was added as triple superphosphate 143
 (N=0 %, P₂O₅=48 %, K₂O=0 %) corresponding to 218 kg P 144
 per ha (500 kg P₂O₅). This supply was high in comparison with 145
 levels generally used because P is very strongly adsorbed by 146
 iron oxides in ultramafic soils, especially in New Caledonia, 147
 where levels of available P are very low (Dubus and Becquer 148
 2001; Jaffré and L’Huillier 2010a, 2010b). Potassium was 149 Q4
 added as sulfate of potash (K₂O=50 %) at 200 kg K₂O per 150
 ha for all treatments, so that the potassium deficiency could not 151
 act as a limiting factor. An irrigation system consisting of a 152
 network of drip lines controlled by solenoid valves was 153
 established to standardize watering and support the develop- 154
 ment of plants. This automated system delivered 0.5 L of water 155
 per day to each plant, except on rainy days. 156
- Plant sampling and chemical analysis** 157
- Two years after planting, roots and leaves of three plants per 158
 plot were sampled (15 plants per treatment). Whole plants 159
 were taken randomly from the experimental layout, roots 160
 were washed several times separately from leaves to remove 161
 soil particles, and plants were dried at 60 °C for 3 days. The 162
 dry weight of both roots and leaves was measured, and then, 163
 each plant part was ground to determine total N (Kjeldahl), 164
 total P, and total Ni by inductively coupled plasma optical 165
 emission spectroscopy (ICP-OES; Optima 3300 DV, 166
 PerkinElmer) at the chemistry laboratory of L’Institut de 167
 recherche pour le développement (IRD) (Laboratoire des 168 Q5
 Moyens Analytiques (LAMA) IRD, Noumea). 169 Q6

170 Root sampling and assessment of mycorrhizal colonization

171 For each treatment, fine roots of the 15 sampled plants were
 172 taken to assess mycorrhizal colonization following staining
 173 with Trypan blue (Koske and Gemma 1989 modified). Root
 174 fragments were placed in 10 % KOH at 90 °C for 90 min; a
 175 few drops of hydrogen peroxide (H₂O₂) were added after 1 h
 176 to clear the brown color. The roots were then rinsed in
 177 distilled water and stained with Trypan blue (15 min at
 178 90 °C). For each plant, at least 25 root segments of 1–
 179 2 cm length were mounted in 10 % glycerol on glass slides
 180 and observed under an optical microscope. A root segment
 181 was considered to be mycorrhizal when arbuscules, vesicles,
 182 or intracellular hyphal coils were clearly identified. The
 183 intensity of arbuscular mycorrhizal colonization, i.e., mycor-
 184 rhizal intensity (M %), was determined according to Trouvelot
 185 et al. (1986). $M (\%) = \frac{95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1}{\text{total number of root fragments}}$ where n₅ is the
 186 number of root fragments with more than 90 % root coloni-
 187 zation, n₄ between 90 and 50 % root colonization, n₃ between
 188 50 and 10 % root colonization, n₂ between 10 and 1 % root
 189 colonization, and n₁ less than 1 % root colonization.
 190 Arbuscule abundance (A %) was also estimated using the
 191 same formula, but considering only the presence of arbuscules
 192 instead of global mycorrhizal colonization.

193 Soil sampling and chemical analysis

194 A volume of 1,500 cm³ of soil was collected near the plants,
 195 at 0–30 cm depth, for soil analyses. Three samples were
 196 collected at different locations in each plot and mixed to
 197 obtain a composite sample representative of the plot. The
 198 samples were air dried immediately after collection and
 199 sieved through a 2-mm mesh before analysis at the chemis-
 200 try laboratory of IRD (LAMA IRD, Noumea). Total and
 201 available P (Olsen) were measured by ICP-OES 3 months,
 202 1 year, and 2 years after planting.

203 Statistical analysis

204 Data, expressed as mean and standard deviation of the mean,
 205 were compared using a variance analysis with the statistical
 206 software V2.0 beta Kiplot 13. Data were checked for normal
 207 distribution. One-way ANOVA including the Tukey test was
 208 carried out for each parameter separately and a total correlation
 209 test to analyze the correlations between the parameters studied.

210 **Results**

211 P and N fertilization clearly improved growth of *C. comosa*
 212 and plant biomass in phosphate- and nitrogen-fertilized
 213 plots was more than three times greater than in the non-
 214 fertilized control plot (Table 1). However, leaf dry weight

did not differ between the fertilization treatments due to the
 high variability of the values. P concentration in roots was
 significantly higher in P-treated plots than in non-fertilized
 plots. The P concentrations in leaves were similar in all the
 treatments, but the amount of P absorbed per plant (P
 content) was nearly three times higher in plots fertilized
 with P and N. N concentrations in roots and leaves did not
 vary according to the fertilization treatment, but plants
 absorbed generally more N in plots in which N and P were
 added. No differences were observed for Ni concentrations
 in roots and leaves between the four treatments.

The average total P in soil at the beginning of the exper-
 iment was 154 mg kg⁻¹, but only 1.8 mg kg⁻¹ was available.
 After 1 year, these values decreased or were maintained at
 the same level in non-fertilized plots (127 mg kg⁻¹ of total
 P; 3.7 mg kg⁻¹ of available P) whilst the P-fertilized plots
 (N+P+, N–P+) showed significantly higher levels, in particu-
 lar for available P with concentrations four to seven times
 higher than in control non-fertilized plots (Table 1). There
 were no significant differences between P- and N-fertilized
 plots (N+P+) and those that only received P (N–P+). After
 2 years, the values of total and available P were not mark-
 edly different from those obtained after 1 year.

The values for mycorrhizal intensity were very low for
 plants in the control plots (2.9 %), but the addition of P greatly
 increased these values to 18.2 and 21.2 % for the N–P+ and
 N+P+ treatments, respectively (Table 1). P fertilization also
 stimulated arbuscule production in mycorrhizal roots (values
 about four times higher than the control). Correlations be-
 tween mycorrhizal and plant nutrition parameters are
 presented in Table 2. There were no significant correlations
 between *C. comosa* plant growth and mycorrhizal coloniza-
 tion. Multiple regression analysis was also performed and did
 not reveal any significant additive effects (multiple correla-
 tions), particularly between growth parameters, phosphate
 fertilization, and mycorrhizal colonization. P concentrations
 in leaves and in roots were highly positively correlated to
 mycorrhizal intensity and arbuscule abundance ($P < 0.05$) in-
 dicating that plants with the highest level of mycorrhizal
 colonization absorbed more P. Mycorrhizal intensity and
 arbuscule abundance were also generally positively correlated
 to total and available P in soil ($P < 0.05$), at the beginning of
 the experiment and after 1 or 2 years (Table 2).

N concentration in roots (but not N concentration in
 leaves) was also positively correlated to mycorrhizal param-
 eters. No correlations were observed between mycorrhizal
 colonization and Ni concentration in leaves and roots.

Discussion

Many plant species endemic to New Caledonia's ultramafic
 maquis are characterized by low growth rates. This is

Table 1 Growth, nutrition, and mycorrhizal colonization characteristics of *C. comosa* plants grown in field, with or without mineral fertilization

	N-P-	N-P+	N+P-	N+P+	
t1.2					
t1.3	Total dry weight (g plant ⁻¹)	27.9±8.9 a	48.1±31.9 ab	45.3±31.7 ab	96.5±49.0 b
t1.4	Leaves dry weight (g plant ⁻¹)	15.4±5.7 a	27.6±18.3 a	31.1±21.1 a	37.83±25.5 a
t1.5	P in leaves				
t1.6	mg kg ⁻¹	220±28 a	288±40 a	199±40 a	269±77 a
t1.7	mg plant ⁻¹	3.4±1.2 a	7.5±4.4 ab	6.3±5.5 ab	9.8±6.9 b
t1.8	P in roots				
t1.9	mg kg ⁻¹	136±10 a	207±51 b	120±15 a	237±64 b
t1.10	mg plant ⁻¹	0.9±0.5 a	2.3±0.9 ab	0.9 ±0.6 a	6.6±2.5 b
t1.11	N in leaves				
t1.12	g kg ⁻¹	8.9±0.3 a	8.2±0.9 a	8.2±1.1 a	7.9±1.2 a
t1.13	mg plant ⁻¹	138.1±50.3 a	215.2±134.9 a	258.5±196.8 a	293.1±191.4 a
t1.14	N in roots				
t1.15	g kg ⁻¹	3.3±0.3 a	3.8±0.9 a	3.8±0.4 a	3.8±1.7 a
t1.16	mg plant ⁻¹	21.9±11.9 a	44.4±21.5 ab	26.9±15.1 a	107.2±54.4 b
t1.17	Ni in leaves (mg kg ⁻¹)	25.8±8.1 a	28.6±6.3 a	26.0±3.1 a	23.0±5.2 a
t1.18	Ni in roots (mg kg ⁻¹)	196.2±48.1 a	231.2±99.4 a	206.6±42.4 a	246.0±66.0 a
t1.19	Total P in soil after 3 months (mg kg ⁻¹) ^a	243.2±123.7 a	230.9±85.0 a	189.4±107.3 a	177.4±161.3 a
t1.20	Available P in soil after 3 months (mg kg ⁻¹) ^a	19.2±19.0 a	13.6±12.2 a	10.2±9.0 a	13.4±12.1 a
t1.21	Total P in soil after 1 year (mg kg ⁻¹)	127.8±10.2 a	231.0±40.9 b	114.1±20.8 a	291.1±83.8 b
t1.22	Available P in soil after 1 year (mg kg ⁻¹)	3.7±1.3 a	15.6±5.9 b	4.2±1.3 a	27.5±14.8 b
t1.23	Total P in soil after 2 years (mg kg ⁻¹)	167.2±22.3 a	313.4±114.5 b	170.0±25.2 a	249.8±107.3 ab
t1.24	Available P in soil after 2 years (mg kg ⁻¹)	3.7±1.3 a	22.9±18.9 b	3.2±1.3 a	16.9±13.6 b
t1.25	Mycorrhizal intensity (M %) after 2 years	2.9±2.7 a	18.2±15.5 b	5.4±5.4 a	21.2±9.5 b
t1.26	Arbuscule abundance (A %) after 2 years	1.0±1.0 a	4.7±4.2 b	1.9±1.5 ab	3.9±1.5 b

^a Just before the addition of NPK and before planting, total P in soil was 154.7 mg kg⁻¹ and available P in soil was 1.8 mg kg⁻¹. For each parameter, means with different letters are significantly different (Tukey–Kramer test, *P*<0.05)

Table 2 Total correlations (Pearson correlation coefficient) between mycorrhizal parameters in the one hand and plant growth, plant nutrition parameters, and P contents in soil in the other (all treatments taken together, 60 values)

		Mycorrhizal intensity, M (%)	Arbuscule abundance, A (%)	
t2.2				
t2.3	Plant parameters	M (%)	1	0.833
t2.4		Total dry weight	-0.149	-0.82
t2.5		Leaves dry weight	-0.231	-0.146
t2.6		P concentration in leaves	0.399**	0.348**
t2.7		P concentration in roots	0.634***	0.450***
t2.8		N concentration in leaves	0.060	0.084
t2.9		N concentration in roots	0.553***	0.332**
t2.10		Ni concentration in leaves	-0.169	-0.022
t2.11		Ni concentration in roots	-0.40	0.154
t2.12	Soil parameters	Total P in soil after 3 months	0.336**	0.240
t2.13		Total P in soil after 1 year	0.370**	0.313*
t2.14		Total P in soil after 2 years	0.331**	0.404**
t2.15		Available P in soil after 3 months	0.324*	0.132
t2.16		Available P in soil after 1 year	0.301*	0.299*
t2.17		Available P in soil after 2 years	0.169	0.270*

Multiple regression analysis was also done and did not reveal significant additive effects. Significant correlations are in bold

P*<0.05, *P*<0.01, ****P*<0.001 (*n*=60)

265 especially true for *C. comosa*, which can grow with very
 266 low levels of main elements and can be considered as
 267 oligotrophic (Jaffré and L'Huillier 2010a, 2010b). In the
 268 present study, the P concentration in *C. comosa* leaves was
 269 low (0.022 %) and did not increase significantly in fertilized
 270 plots (0.028 %). Available P was strongly deficient in the
 271 ultramafic soil (only 1.8 mg kg⁻¹, before fertilization) in
 272 relation to its pH (5.3) and to the strong adsorption to iron
 273 oxides (Dubus and Becquer 2001; Jaffré and L'Huillier
 274 2010a, 2010b). One year after fertilization, total P values
 275 in soil were about twice than that recorded for non-fertilized
 276 plots and available P was about ten times higher, but
 277 stayed relatively low (15–27 mg kg⁻¹). Under these
 278 conditions, *C. comosa* growth was stimulated by P
 279 supply, particularly when N was also added. The high
 280 variability of the values obtained for different parame-
 281 ters, especially for plant dry weight, can be related to
 282 soil heterogeneity as underlined by Lagrange (2009)
 283 from physicochemical analyses of different samples tak-
 284 en in the studied experimental field.

285 AM fungal propagules are relatively abundant in New
 286 Caledonian ultramafic soils (Perrier et al. 2006; Amir and
 287 Ducouso 2010). It is the case with the soil studied here
 288 which was used as natural inoculum by Lagrange et al.
 289 (2011) and induced a relatively good mycorrhizal coloniza-
 290 tion in sorghum plants ($M=37\%$). The low level of mycor-
 291 rhizal colonization (<3 %) of *C. comosa* plants in non-
 292 fertilized plots could then be considered as an illustration
 293 of the low affinity of Cyperaceae species with AM fungi
 294 (Miller et al. 1999; Wang and Qiu 2006). However, it is
 295 important to point out that mycorrhizal intensity in the *C.*
 296 *comosa* plants increased to 18–21 % in P-amended plots.
 297 This stimulation of mycorrhizal colonization can be related
 298 to P supply, since it was not significantly enhanced by N
 299 when added without P. The concomitant increase in
 300 arbuscule abundance in P-amended plots indicates that the
 301 AM fungi form a functional symbiosis (Brundrett 2009).
 302 Indeed, P concentrations in roots and leaves were highly
 303 positively correlated with mycorrhizal intensity and
 304 arbuscule abundance so that plants having the highest over-
 305 all mycorrhizal colonization absorbed more P, suggesting a
 306 role of the fungal symbionts in P uptake, as for many AM
 307 plant species (Bolan 1991; Ezawa et al. 2002; Smith and
 308 Read 2008). The absence of a non-mycorrhizal control
 309 treatment for each of the four fertilization treatments, in
 310 the context of a field experiment, does not allow a direct
 311 evaluation of the effects of AM fungal colonization on P
 312 uptake by *C. comosa*. Root/shoot ratios are frequently re-
 313 duced by AM fungal colonization, in comparison with non-
 314 mycorrhizal plants (Smith and Read 2008; Veresoglou et al.
 315 2012). This was not the case when AM fungal colonization
 316 increased in *C. comosa* in the P-fertilized plots (root/shoot
 317 ratios 0.811 for N–P– and 0.742 for N–P+). On the contrary,

318 in N- and P-fertilized plots (N+P+), the root/shoot ratio
 319 clearly increased (1.551). However, under these conditions,
 320 it is difficult to distinguish the direct influence of P supply
 321 on the plants from the effect of the higher AM fungal
 322 colonization. Indeed, increased P supply to plants can
 323 induce root proliferation (Drew 1975; Sun et al. 2002;
 324 He et al. 2003). Nevertheless, P uptake efficiency by *C.*
 325 *comosa* plants (total P absorbed per milligram roots)
 326 was improved in plots only fertilized with P (0.344
 327 for N–P–; 0.478 for N–P+), but not in the N- and P-
 328 fertilized plots (0.279 for N+P+) where the increase in
 329 root biomass could have masked the effect of AM fungi
 330 on P absorption. In addition, the efficiency of the my-
 331 corrhizal pathway for P uptake will depend not only on
 332 arbuscule abundance for transfer to the plant, but also
 333 on the amount of extraradical hyphae involved in uptake
 334 from the soil and translocation to roots, which was not
 335 estimated in the present study.

336 The significant positive correlations between mycorrhizal
 337 parameters with the total and available P in soil indicate that
 338 *C. comosa* root colonization by AM fungi is promoted in
 339 soils with the highest levels of P, suggesting that P deficien-
 340 cy in soil reduces the ability of this plant species to form
 341 mycorrhiza. It is well known that high levels of P can inhibit
 342 mycorrhizal colonization in other plants (Marschner 1995),
 343 but the effects of P deficiency on mycorrhizal colonization
 344 have not been clearly demonstrated. Under different condi-
 345 tions, Schubert and Hayman (1986) considered that mycor-
 346 rhizal development in onion plants was optimal at
 347 50 mg kg⁻¹ of available P in agricultural soils, whilst other
 348 studies have reported a relatively high level of mycor-
 349 rhizal colonization of other plants in highly P-deficient
 350 soils (Vivas et al. 2003; Chaudhry et al. 2005; Zangaro
 351 et al. 2008; Pagano et al. 2010). It is clear that strong P
 352 deficiency cannot always be a limiting factor for my-
 353 corrhizal colonization. However, the present results sug-
 354 gest that, under particular conditions like those in
 355 ultramafic soil, strong P deficiency may reduce root
 356 colonization by AM fungi, at least for plant species like
 357 *C. comosa*.

358 Lambers et al. (2009) suggested that mycorrhiza strate-
 359 gies may not be effective for the plant in highly P-deficient
 360 soils and considered that specialized roots, such as cluster or
 361 dauciform roots, in non-mycorrhizal plant species could be
 362 more efficient for P absorption in these conditions. The root
 363 system of *C. comosa* develops dauciform roots that may be
 364 colonized by AM fungi (Lagrange et al. 2011). Dauciform
 365 roots were observed in *C. comosa* in the present field
 366 experiment, without differences in their development be-
 367 tween P-fertilized or non-fertilized plots, indicating that
 368 plants were still under P deficiency stress (Neumann
 369 and Martinoia 2002), probably because of the very
 370 strong adsorption of added P to iron oxides in this type

371 of soils (Dubus and Becquer 2001; Jaffré and L’Huillier
372 2010a, 2010b).

373 It is not clear whether the increase in *C. comosa* biomass
374 in fertilized plots is only related to P and N supply or if AM
375 fungal activity contributed to this effect. No multiple corre-
376 lations were found between these parameters. The absence
377 of a significant correlation between mycorrhizal coloniza-
378 tion and plant growth, as noted here, is relatively frequent
379 (Smith and Smith 2010) and can be related to the high
380 variance inside each group of values, reflecting interactions
381 with a large number of variables, as is frequently the case in
382 field experiments.

383 In this study, nickel concentration in leaves and roots of
384 *C. comosa* was not negatively correlated to mycorrhizal
385 colonization, which does not support the results of
386 Lagrange et al. (2011) who found that Ni concentration in
387 roots of *C. comosa* grown under greenhouse conditions was
388 reduced by inoculation with AM fungi. It is possible that the
389 high variability in values under field conditions could mask
390 the protective effect of AM against nickel toxicity which has
391 been reported also for other plant species endemic to New
392 Caledonia’s ultramafic maquis (Amir and Ducouso 2010;
393 Amir et al. 2012).

394 In conclusion, the present study shows that the mycor-
395 rhizal status of the sedge species *C. comosa* varies in rela-
396 tion to available P concentration in ultramafic soil. Brundrett
397 (2009), discussing the mycorrhizal status of the Cyperaceae
398 family, proposed different hypotheses to explain the variable
399 reports concerning this status (NM-AM). One of them is that
400 AM fungi can be found at low frequencies in Cyperaceae
401 plant roots, but they do not form arbuscules and are not
402 functional, behaving as endophytic or saprophytic organ-
403 isms. This hypothesis is in contradiction with the present
404 and previous observations (Lagrange et al. 2011). The sec-
405 ond hypothesis is that Cyperaceae are potentially AM, but
406 often occur in mycorrhiza-suppressive habitats. The present
407 results clearly support this hypothesis, since *C. comosa* is
408 generally weakly mycorrhizal in natural conditions (Perrier
409 et al. 2006; Lagrange et al. 2011) with very low levels of soil
410 P, but its mycorrhizal status is clearly AM in P-fertilized
411 soil. The positive effect of moderate P fertilization on my-
412 corrhizal colonization of *C. comosa* could be useful for
413 ecological restoration of degraded mining areas. Indeed, this
414 plant is one of the species most used for this purpose, and
415 current experiments aim at determining the optimal doses of
416 mineral P which may stimulate mycorrhizal colonization as
417 well as plant growth.

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

- Q1. Please check captured e-mail address if presented appropriately.
- Q2. Please check if the affiliations are presented correctly.
- Q3. The term urea-methylene was changed to "methylene urea." Please check if appropriate.
- Q4. The citation "Jaffré and L'Huillier 2010" (original) has been changed to "Jaffré and L'Huillier 2010a, 2010b." Please check if appropriate.
- Q5. IRD was spelled out as L'Institut de recherche pour le développement. Please check.
- Q6. LAMA was spelled out as Laboratoire des Moyens Analytiques. Please check.
- Q7. Please check if the data in Tables 1 and 2 are presented correctly.
- Q8. Veresoglou et al. 2012, Pagano et al. 2010, Zangaro et al. 2008, Chaudhry et al. 2005, and Vivas et al. 2003 are cited in text but not given in the reference list. Please provide details in the list or delete the citations from the text.
- Q9. Please provide updated information. If possible, please advise the DOI number if this reference is still for publication or in press.
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