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Article

Pure coconut coir as a sustainable nursery growing media for seedling production of ecologically diverse *Quercus* species

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Abstract: Peat, a non-sustainable resource, is still predominately used in forest nurseries. Coconut coir may provide an alternative, renewable, and reliable growing media but few studies have evaluated this media type in forest nurseries. We assessed the influence of pure coir in combination with various fertilization regimes on the growth and physiology of three ecologically diverse *Quercus* species seedlings (*Q. robur*, *Q. pubescens*, *Q. ilex*) during nursery cultivation. Seedlings were grown using peat and pure coir in combination with 3 fertilization treatments (standard, K enriched, P enriched). Data were collected for: 1) growth and physiological traits; 2) detailed above- and below-ground morphological traits by destructive analysis; and, 3) NPK content in leaves, shoot and roots, and in growing media following cultivation.

Peat and coir in combination with the various fertilization treatments affected above- and below-ground morphology and, to a lesser extent, physiological traits of *Quercus* seedlings. Large effects of substrate occurred for most morphological variables, with peat more effective than coir in all studied species. Fertilization also produced significant differences. The effect of K-enriched fertilization on plant growth was clear across the three species and the two growing media. P-enriched fertilization in peat was the only combination that promoted a higher amount of this element in the tissues at the end of cultivation.

Despite the relatively smaller size, seedlings produced in coir were in compatible with standard *Quercus* forest stocktypes size, and showed a proportionally higher root system development and fibrosity. Our results suggest that coir can be used as an alternative substrate to grow *Quercus* species seedlings, and that fertilization can offset coir deficiencies in chemical properties. Because several functional traits drive planting performance under varying environmental conditions according to the Target Plant Concept, coir may thus serve as an acceptable material for seedling cultivation in some cases.

Keywords: forest nursery stock; coconut fiber; peat; seedling morphology; seedling physiology; growing media.

47

48 **1. Introduction**

49

50 Forest seedling field performance is related to target plant morphological and physiological
51 characteristics [1–3]. Development of morpho-physiological traits, in turn, is strongly influenced by
52 nursery practices [1,4–7]. In container plant production, the effectiveness of the growing medium is
53 important to seedling quality [8,9]. An effective substrate should sustain a favourable balance
54 between air porosity and water holding capacity, promoting root development and nutrient uptake
55 [9,10]. Additionally, growing media must have a high cation exchange capacity and be economically
56 viable [11].

57 Sphagnum peat moss (*Sphagnum* spp.), generally known as peat, is commonly and
58 predominantly used in Europe and North America [9,12–15]. Peat production globally is about 28 Mt
59 [15] and peatlands used for growing media cover about 2000 km² [16]. The effectiveness of peat is
60 related both to material performance and economic aspects [17]. Despite a low re-wetting capacity
61 [18], peat is a light, low bulk density material that contains low nutrient content but has a very high
62 cation exchange capacity (CEC) [8]), and can adsorb fertilizer nutrients easily; thus, plant nutrition
63 can be controlled during cultivation [19]. Moreover, peat requires relatively few post-harvest
64 treatments and additives to be used effectively [9]. Nevertheless, concerns have been raised about
65 environmental impacts of peat extraction [20–22] in relation to: a) the fragility of many peatland
66 ecosystems [16,23–25]; b) their function as a C sink, whose drainage and exploitation increases C
67 emissions [22,26]; and c) the non-sustainable length of the natural processes of peat production
68 [24,25,27]. Environmental concerns in Europe promoted actions to preserve peatlands as ecosystem
69 as well as to reduce C emissions [17,28]. The growing pressure on producers, retailers and growers,
70 especially in the horticulture sector, has led to an increasing demand of alternative, renewable, and
71 reliable growing media [29,30]. Coir (or coconut fiber) has been tested as an alternative to peat in
72 horticulture [31], and is the most used alternative in this sector [14,32]. Coir is made of husk and short
73 fibers from the nut mesocarp of *Cocos nucifera*, which are a waste product of the coconut industry,
74 coming mainly from Philippines, Indonesia, Sri Lanka, Malaysia and Thailand. Coir is a renewable
75 and largely available resource, and 25% of over 50 million tons of coconut produced annually are
76 waste [33]. Coir provides a favourable balance between air and water, similar to peat [9], and a higher
77 re-wetting capacity than peat [34]. However, coir has higher pH and lower cation exchange capacity
78 (CEC) than peat [8]. In addition, to be effective as a soilless growing medium, coir needs to be further
79 processed, which can lead to a low standardization of biological, chemical or physical properties of
80 the material as compared to peat [9]. In particular, when it is produced in coastal marine areas, coir
81 has to be treated to reduce toxic levels of sodium and potassium [35]. Such procedures increase coir
82 production costs. According to Schmiliwski [32], coir is the third most common growing medium
83 used by nurseries in horticulture after peat (which covers more than 2/3 of the market) and mineral
84 substrates (such as vermiculite, perlite, rockwool, and others). According to recent reports [28,36], in
85 Netherlands and in Italy, the use of coir is increasing.

86 Seedling physiological and morphological traits can also be strongly altered by fertilization
87 during nursery cultivation, potentially interacting with substrate chemical properties. This may
88 affect plenty of attributes, such as biomass accumulation and allocation to shoot- and root-system
89 [37,38], leaf morphology and physiology [7,39], root-system architecture and functionality [7,40], and
90 xylem conductance [41] with effects on survival and field performance [42–44]. Hence, by altering
91 fertilization it may be possible to affect seedling quality [45,46] and the performance of transplanted
92 seedlings [47]. Seedling nutrient availability can influence the amount of reserves available for
93 remobilization after planting [44,48]. While many studies have investigated the effects of nitrogen
94 fertilization on forest tree seedlings, less is known about phosphorous and potassium [43].
95 Phosphorus is an immobile soil resource whose availability is linked to morpho-functional traits of
96 the root system [49–53], and, consequently, it may have indirect effects on seedling post-planting
97 survival, nutrient uptake and growth, drought resistance, and more in general, stress resistance

98 [40,54–56]. Less information is available for forest species about the effect of K, which is an element
99 directly related to a plethora of physiological processes, including, among others, cellular turgor,
100 stomatal conductance, and photosynthesis, which can influence drought resistance [57–60].

101 Although coir was introduced in horti-flori-fructiculture nursery production about two decades
102 ago, and extensive literature covers its productive and economic aspects in these systems [9], far less
103 information is available for the forest nursery sector [i.e., [11,61,62]. Moreover, coconut fiber is often
104 tested as a component at different proportions of a soilless growing media mixture with other
105 materials, complicating assessment of coir as single component [9], especially in relation to
106 deficiencies in its chemical properties (i.e. CEC). In this sense, the use of controlled release fertilizer
107 (CRF) with coconut fiber could help to offset mentioned low CEC. In this study, we focused on
108 *Quercus* species widely used in the Mediterranean region for reforestation, afforestation and forest
109 restoration projects [63–66]. The species were chosen according to different ecological adaptations,
110 particularly in relation to Mediterranean and/or arid climates [67]: from the most well adapted to a
111 Mediterranean climate with dry summers, *Q. ilex* (holm oak), to the intermediate *Q. pubescens*
112 (pubescent or downy oak), to the less Mediterranean adapted, *Q. robur* (pedunculate oak), which
113 prefers a temperate climate without a dry season, is widespread across Europe including the
114 Mediterranean region, and is one of most common species for multi-purpose hardwood forest
115 plantings in Europe [68,69]. We aimed to improve the knowledge of the influence of pure coir on the
116 growth and physiology of these species during nursery cultivation. Coir was combined with three
117 different fertilization treatments (nursery standard, enriched in K, enriched in P) to assess combined
118 effects on seedlings in relation to: height, morphological traits, physiological traits and NPK content
119 both in shoot- and root-system. Specifically, we addressed the following questions: (1) Is pure coir
120 effective in growing forest seedlings to be used in forest plantation projects? (2) Which traits are
121 mainly influenced by this renewable and more sustainable growing medium? (3) Is it possible to
122 offset coir deficiencies in chemical properties by fertilization? (4) Did the studied species, which have
123 different leaf habits despite genetic proximity, respond differently to fertilization and substrate
124 treatments?

125

126 2. Materials and Methods

127

128 2.1 Nursery stock cultivation

129

130 The nursery stock was grown in 2017 in a central Italy nursery (43°55'31.4"N, 10°53'09.1"E, 85 m
131 a.s.l.), using multi-pot containers (HerkuPlast QuickPot) with 12 cavities of 650 cm³ each (frustum of
132 pyramid shape; top width 76 mm, bottom width 17 mm, depth 180 mm).

133 Peat substrate (*Pe*), considered as control, was made of 70% coarse Baltic peat moss (0–40 mm
134 particles size) and 30% pumice (5–8 mm), which was added to avoid excessive compaction of pure
135 peat. Main traits of mixture were: pH 5.7, EC 0.43 dS/m; bulk density 298 kg/m³, and porosity 86.9%.
136 Coir (*Co*) was composed of 30% fiber (fibrous material that constitutes the thick mesocarp of the nut)
137 and 70% pith (fine residual material), with pH 5.2, EC 0.38 dS/m; bulk density 122 kg/m³, porosity
138 92.5%. The week before sowing, the cavities were uniformly filled with growing media to 0.5 cm
139 beneath the top.

140 Standard fertilization (*St*), a CRC fertilizer widely used in Italy to commercially grow oak
141 seedling nursery stock, was considered as control, and it was compared to two alternative
142 commercial fertilization formulas: enriched in potassium (*K*) or phosphorus (*P*). *St* formula was
143 Osmocote Exact Standard 12-14 months NPK 15-9-11 plus micronutrients at 3 kg·m⁻³. P enriched
144 substrate contained an addition of 19% P₂O₅ fast release (single superphosphate, SSP, 2 kg·m⁻³) to
145 control, while K enriched substrate was obtained by 4 kg·m⁻³ of Osmocote Exact Standard High K 12-
146 14 months NPK 11-11-18 plus micronutrients. N, P, and K amount per seedling is showed in Table
147 S1.

148 A total of 6 treatments combinations per species (2 substrates x 3 fertilizations) were included in
149 the experimental trial.

150 Seed was obtained from the National Center for Biodiversity (Italy); seed provenances were the
151 following for *Q. robur*, *Q. pubescens*, and *Q. ilex*, respectively: Bosco Fontana (43°55'31.4"N,
152 10°53'09.1"E), Ponte di Veja (45°36'27.4"N, 10°58'15.9"E), Torri del Benaco (45°36'59.1"N, 10°41'50.0"E).
153 The 1,000-seed weight and germination of the three seed lots (according to ISTA procedures) were
154 6.0 kg and 80% for *Q. robur*, 2.4 kg and 68% for *Q. pubescens*, 3.2 kg and 83% for *Q. ilex*, resp. From
155 October 2016 to April 2017, the acorns were stored at 3 ± 0.5 °C in moist sand to simulate normal
156 overwintering and to prevent acorn germination before the experiment started. In March 2017, the
157 seeds were moved outside to stimulate germination, and then the pre-germinated acorns (0.5 cm
158 maximum radicle length) were sown in multi-pots placed under a tunnel protected by transparent
159 plastic film that was removed at mid-May. Seedlings were irrigated daily by sprinklers following
160 seedling evapotranspirative demands (i.e. spring: 5 l·m⁻² in 6 min; summer and autumn: 40 l/m² and
161 20 l/m², resp. in 24 min). Environmental conditions (air temperature and humidity) were monitored
162 by a weather station (inside the tunnel) and substrate moisture was assessed once a week (6 pots per
163 stocktype, Soil moisture meter PCESMM1, PCE Instr. Corp.).

164

165 2.2 Data collection

166

167 Emergence and height were measured weekly from the end of March until September. Here,
168 final height data are presented. During the season, physiological traits, such as Chlorophyll content
169 (SPAD-502, Konica-Minolta Sensing Europe B.V) and Chlorophyll fluorescence (ChlF, by Handy
170 PEA, Hansatech, UK) were measured monthly on a sample of three fully expanded leaves on 9
171 seedlings per stocktype in dark-adapted leaves (for at least 40 minutes) during the growing season
172 (Jun, Jul, Aug). The PSII functionality was described by F_v/F_m (ratio of Variable to Maximum
173 Fluorescence) to Strasser et al. [70]. Results related to physiological traits were only for August, the
174 data closer to plant lifting.

175 Macro-element (N, P, K) were assessed in October (before leaf abscission) on 12 seedlings per
176 stocktype ($S \times F$) per species, merging 2 plants of the same multi-pot, by Nutrilab (Universidad Rey
177 Juan Carlos, Madrid, Spain). Briefly, analysis of leaf, stem and root N and P concentration of these
178 samples was done by the standard Kjeldahl method, while K concentration was determined using a
179 perchloric acid extraction. Substrate analysis at the end of the nursery cultivation was performed by
180 Denetra snc (Pescia, Italy) on 9 seedlings per stocktype ($S \times F$) per species. Concurrently, seedling
181 morphological traits were assessed, using 20 destructively sampled seedlings per treatment
182 combination (360 in total). Height (H), number and dry biomass of leaves (L_n and L_b), root-collar
183 diameter (R_cD) and dry biomass of the shoot (stem) were measured. Root-system was assessed by
184 main root (tap-root) dry biomass (MR_b); first order lateral roots ($FOLR$) dry biomass ($FOLR_b$) for three
185 diameter classes (<1 mm, 1-5 mm, >5 mm) measured at junction with the tap root, root-system volume
186 (by immersion) and dry weight and density (R_v , R_b and R_d resp.). Shoot to root ratio (S/R), H/R_cD ,
187 and Specific Leaf Area (SLA) were also calculated.

188

189 2.3 Statistical analysis

190

191 A randomized complete block design (3 blocks) was used. Each block included the 6 randomized
192 $S \times F$ combinations per species; each combination comprised 48 sowed cavities (over 4 multi-pots),
193 for a total of 2592 sowed containers. In a preliminary multifactorial ANOVA (model, $Y_{ijklm} = \mu + \text{Block}_i$
194 $+ \text{Date}_j + \text{Substrate}(S)_k + \text{fertilization}(F)_l + S \times F \text{ interaction}_{kl} + \text{error}_{ijklm}$), the block effect was not
195 significant for any morphological variables, highlighting homogeneous growing conditions.
196 Multifactorial ANOVA was performed, separately for each species to avoid complex higher-level
197 interactions, considering substrate (S), fertilization (F), and their interactions ($S \times F$) as source of
198 variation. In case of significant results ($p \leq 0.05$), Tukey post hoc test was used for multiple
199 comparisons ($\alpha = 0.05$) to highlight homogenous groups within species. In this study, we present
200 results related to substrate (S), fertilization (F), and their significant interactions ($S \times F$). To provide
201 information about the traits of the studied stocktypes (combinations $S \times F$), Tukey test results among

202 combinations are shown in supplementary material. StatSoft Statistica 11 (Tulsa, Oklahoma, USA)
 203 was used to process all data.

204

205 3. Results

206

207 3.1 Emergence, growth and physiological traits during nursery cultivation

208

209 Final emergence was not affected by either factor in the three studied oaks. In *Q. ilex* seedlings
 210 emergence started between 3 and 4 weeks later than in the two species (data not shown). In all species,
 211 substrate affected height beginning relatively early, from June (from May in *Q. robur*, data not
 212 shown), and seedlings in *Pe* where higher than in *Co* (Table 1); in all species, fertilization effect
 213 occurred later (September in *Q. robur*, July in *Q. pubescens*, July in *Q. ilex*, data not shown), and K-
 214 enriched fertilization promoted taller seedlings (Table 1). As a result, at the end of the growing
 215 season, the tallest stocktype was grown in *Pe-K* in all species with different gaps with the stocktypes
 216 in *Coir*, and generally, seedlings grown in *Co-St* performed worse (Table S2). Interaction was not
 217 significant in any case, excluding *Q. robur* in August and *Q. ilex* in August and September.

218

219 **Table 1.** Multifactorial ANOVA and Tukey post hoc test results ($p \leq 0.05$ in bold) for seedling morphological traits
 220 (mean \pm SD) at the end of the season ($N_{\text{obs}} = 20$ seedlings per stocktype per species). Source of variation: substrate
 221 (*S*), fertilization (*F*), and their interaction (*SxF*). Capital and lowercase letters indicate homogeneous groups for
 222 *S* and *F*, resp. Variables: *H* (height); *RcD* (root collar diameter in mm); *H/RcD* (seedling taper); *Rv* (root-system
 223 volume in cm^3); *Rd* (root-system density= Rb/Rv in g cm^{-3}); *S/R* (shoot-root ratio); *SLA* (specific leaf area, in $\text{cm}^2 \text{g}^{-1}$);
 224 *Ln* (number of leaves); *Lb* (leaf dry biomass in g).

<i>Q. robur</i>	<i>S</i>	<i>F</i>	<i>SxF</i>	<i>Pe</i>	<i>Co</i>	<i>St</i>	<i>P</i>	<i>K</i>
<i>H</i>	<0.0001	0.0108	0.8213	57.6 \pm 10.8 B	45.8 \pm 10.3 A	48.3 \pm 11.4 a	51.5 \pm 12.2 ab	55.4 \pm 11.8 b
<i>RcD</i>	0.2265	0.0006	0.6354	11.2 \pm 1.9	10.8 \pm 1.7	10.1 \pm 1.8 a	11.2 \pm 1.8 ab	11.6 \pm 1.6 b
<i>H/RcD</i>	<0.0001	0.4976	0.9521	52.6 \pm 11.3 B	42.9 \pm 9.1 A	48.4 \pm 12.2	46.1 \pm 9.8	48.6 \pm 11.9
<i>Rv</i>	0.0143	0.0001	0.1355	28.0 \pm 9.2 B	24.6 \pm 7.8 A	21.0 \pm 6.0 a	26.9 \pm 8.0 b	31.0 \pm 8.8 b
<i>Rd</i>	0.3264	0.0040	0.0711	0.43 \pm 0.07	0.44 \pm 0.09	0.47 \pm 0.10 b	0.42 \pm 0.06 ab	0.41 \pm 0.06 a
<i>S/R</i>	<0.0001	0.0419	0.6985	0.65 \pm 0.14 B	0.49 \pm 0.15 A	0.53 \pm 0.17 a	0.57 \pm 0.14 ab	0.61 \pm 0.18 b
<i>SLA</i>	0.0016	0.0120	0.5480	156.1 \pm 29.2 B	142.1 \pm 18.1 A	147.0 \pm 20.2 ab	142.2 \pm 32.0 a	157.9 \pm 19.3 b
<i>Ln</i>	0.0156	<0.0001	0.7843	56.6 \pm 26.0 B	47.2 \pm 19.7 A	41.6 \pm 13.2 a	48.0 \pm 22.0 ab	66.1 \pm 26.2 b
<i>Lb</i>	<0.0001	<0.0001	0.2137	4.5 \pm 1.5 B	3.4 \pm 1.3 A	3.2 \pm 1.1 a	4.0 \pm 1.3 b	4.7 \pm 1.6 b
<i>Q. pubescens</i>	<i>S</i>	<i>F</i>	<i>SxF</i>	<i>Pe</i>	<i>Co</i>	<i>St</i>	<i>P</i>	<i>K</i>
<i>H</i>	<0.0001	0.0028	0.4337	36.8 \pm 11.5 B	22.4 \pm 8.2 A	27.9 \pm 10.7 ab	27.0 \pm 11.1 a	33.9 \pm 14.0 b
<i>RcD</i>	0.0003	0.0024	0.4202	10.7 \pm 1.9 B	9.4 \pm 2.2 A	9.7 \pm 1.9 ab	9.4 \pm 2.3 a	10.9 \pm 2.0 b
<i>H/RcD</i>	<0.0001	0.5218	0.5139	34.8 \pm 10.3 B	24.2 \pm 7.2 A	28.9 \pm 10.6	28.9 \pm 9.5	30.8 \pm 11.1
<i>Rv</i>	<0.0001	0.3988	0.0951	25.8 \pm 10.5 B	14.9 \pm 6.1 A	20.9 \pm 12.6	19.1 \pm 8.6	21.3 \pm 9.0
<i>Rd</i>	0.0101	0.2732	0.0215	0.41 \pm 0.07 A	0.44 \pm 0.06 B	0.42 \pm 0.08	0.43 \pm 0.06	0.42 \pm 0.05
<i>S/R</i>	<0.0001	0.0001	0.0725	0.43 \pm 0.13 B	0.27 \pm 0.11 A	0.33 \pm 0.11 a	0.31 \pm 0.15 a	0.42 \pm 0.16 b
<i>SLA</i>	0.0600	0.6396	0.7851	104.6 \pm 13.4	100.6 \pm 8.7	101.4 \pm 9.0	103.8 \pm 13.9	102.7 \pm 11.2
<i>Ln</i>	<0.0001	0.0232	0.4068	60.5 \pm 27.2 B	34.9 \pm 15.1 A	46.5 \pm 29.7 ab	41.7 \pm 20.5 a	54.9 \pm 24.0 b
<i>Lb</i>	<0.0001	0.0192	0.4390	4.3 \pm 1.5 B	2.1 \pm 1.1 A	3.1 \pm 1.7 ab	2.9 \pm 1.4 a	3.7 \pm 1.9 b
<i>Q. ilex</i>	<i>S</i>	<i>F</i>	<i>SxF</i>	<i>Pe</i>	<i>Co</i>	<i>St</i>	<i>P</i>	<i>K</i>
<i>H</i>	<0.0001	0.0032	0.3332	52.7 \pm 13.1 B	35.0 \pm 10.3 A	40.9 \pm 13.0 a	41.8 \pm 13.3 ab	48.9 \pm 16.7 b
<i>RcD</i>	0.0114	0.1610	0.5929	8.5 \pm 1.1 B	7.8 \pm 1.6 A	8.1 \pm 1.3	7.9 \pm 1.5	8.5 \pm 1.3
<i>H/RcD</i>	<0.0001	0.0386	0.2352	62.7 \pm 15.4 B	45.1 \pm 12.3 A	50.1 \pm 13.4 a	53.6 \pm 15.7 ab	58.0 \pm 19.2 b
<i>Rv</i>	<0.0001	0.0009	0.4449	14.9 \pm 5.0 B	10.7 \pm 4.3 A	10.8 \pm 4.2 a	12.9 \pm 5.4 ab	14.6 \pm 5.1 b

<i>Rd</i>	0.3072	0.1237	0.3875	0.45 ± 0.10	0.43 ± 0.13	0.45 ± 0.13	0.41 ± 0.08	0.46 ± 0.12
<i>S/R</i>	0.0062	0.3922	0.3637	0.71 ± 0.25 B	0.59 ± 0.22 A	0.68 ± 0.24	0.61 ± 0.29	0.67 ± 0.19
<i>SLA</i>	0.0104	0.2676	0.0005	73.6 ± 8.0 B	70.5 ± 5.5 A	73.2 ± 7.6	70.7 ± 7.1	72.2 ± 6.4
<i>Ln</i>	<0.0001	0.0140	0.3323	49.4 ± 15.6 B	30.0 ± 11.1 A	38.6 ± 16.2 ab	36.0 ± 13.6 a	44.5 ± 19.0 b
<i>Lb</i>	<0.0001	0.0040	0.1082	5.2 ± 1.5 B	2.9 ± 1.2 A	3.7 ± 1.5 a	3.9 ± 1.8 ab	4.6 ± 2.0 b

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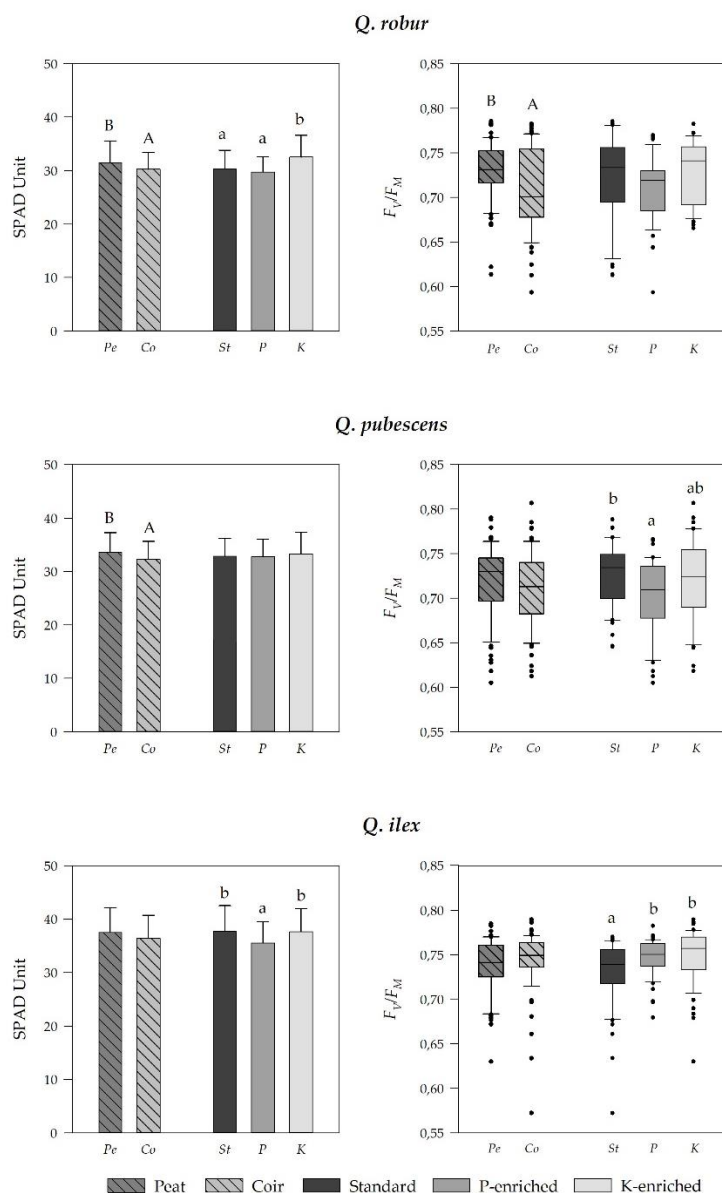
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In general, in all species, in August (Figure 1), both substrate ($Pe > Co$) and fertilization (K generally higher) affected ChlF content, generally with no interactions (data not shown). In *Q. robur* and *Q. pubescens*, seedlings in peat showed higher values of F_v/F_m than in coir (significant in *Q. robur*, Figure 1), and K enriched fertilization generally resulted in higher F_v/F_m values (significant in *Q. ilex*).

Figure 1. Multifactorial ANOVA and Tukey post-hoc test results of F_v/F_m values (box whisker plot) and SPAD units (mean and SD) analyzed for substrate and fertilization ($N_{obs}=81$ for substrate; $N_{obs}=54$ for fertilization) in August. Capital and lowercase letters indicate homogenous groups for substrate and fertilization, respectively. $Pe=$ Peat; $Co=$ Coir; $St=$ Standard fertilization; $K=$ K-enriched fertilization; $P=$ P-enriched fertilization



237

238

239 3.2 Morphological traits

240

241 In *Q. robur*, both substrate and fertilization affected most of the analysed variables without any
 242 interaction (Table 1 and 2), and, generally, *Pe* and K-enriched fertilization were more effective in
 243 promoting seedling growth. For total dry biomass, the differences were as follows: *Pe*>*Co* by +22.6%,
 244 K>*P* and *St* by +13.4% and +36.6%, respectively (Figure 2). Peat also promoted shoot-system and leaf
 245 biomass, *SLA*, as well as *FOLR1-5* and roots total biomass. K-enriched fertilization positively
 246 influenced shoot development, *SLA*, *FOLR1-5* and root system and leaf biomass. *Pe* stimulated
 247 biomass accumulation more in shoot- than in root-system with *S/R* values >0.5 (Table 2), and K-
 248 enriched fertilization promoted higher *S/R* than *St*. Root volume was affected by substrate (*Pe*>*Co*)
 249 and fertilization (*K*>*St*); root density was influenced only by fertilization (*Co*-*St*>*Pe*-*K*).

250 In *Q. pubescens*, substrate affected morphological variables more than fertilization, and,
 251 generally, in the case of significant differences, seedlings in peat and K-enriched fertilization were
 252 better developed (Table 1 and 2, Figure 2). Total biomass in *Pe* was higher than in *Co* by +78.3%, and
 253 the gap between *K* and *St* and *P* was +19.3% and +20.1%, respectively. *Pe* significantly promoted all
 254 biomass variables excluding *FOLR*>5. Fertilization with K promoted biomass growth in the shoot-
 255 system, leaves and in *FOLR1-5* over *St* fertilization. Shoot to root ratio was lower than 0.51 in all
 256 stocktypes (Table 1) and was affected by both substrate (*Pe*>*Co*) and fertilization (*K*>*P* and *St*). Root
 257 volume of *Q. pubescens* seedlings raised with peat was higher than that of coir, while the opposite
 258 occurred for root density.

259 As per previous species, in the case of *Q. ilex*, substrate affected the majority of the analysed
 260 variables (with a *Pe*>*Co* pattern), and generally, when fertilization was significant, seedlings grown
 261 in K-enriched fertilizer had higher values (Tables 1 and 2, Figure 2). Total biomass was affected by
 262 both factors (*Pe* +63.5% than *Co*; *K* +36.2% and +50.2% than *P* and *St*, respectively). Similar differences
 263 (*Pe*>*Co*) occurred for shoot-system and leaf biomass, main root, *FOLR*<1, *FOLR1-5*, and root biomass.
 264 Fertilization affected leaf, main root, *FOLR1-5*, and root biomass (*K*> *P* and *St*). *S/R* ratio was affected
 265 only by substrate (Table 2), both in *Co* and *Pe* it resulted higher than 0.50 (Table 2). Root volume was
 266 affected by both substrate (*Pe*>*Co*) and fertilization (*K*>*P* and *St*).

267

268 **Table 2.** *p* values from multifactorial ANOVA test results: (*p* < 0.05 in bold) for seedlings biomass allocation
 269 (*N*_{obs} =20 per stocktype per species). Sources of variation: substrate (*S*), fertilization (*F*), and their interaction
 270 (*Sx**F*). *Sb*: shoot-system biomass; *MRb*: main root biomass; *FOLRb*: First Order Lateral Root biomass split by
 271 diameter class (<1 mm, 1-5 mm, >5 mm); *Rb*: total root-system biomass; *Sb* + *Rb*: total seedling biomass.

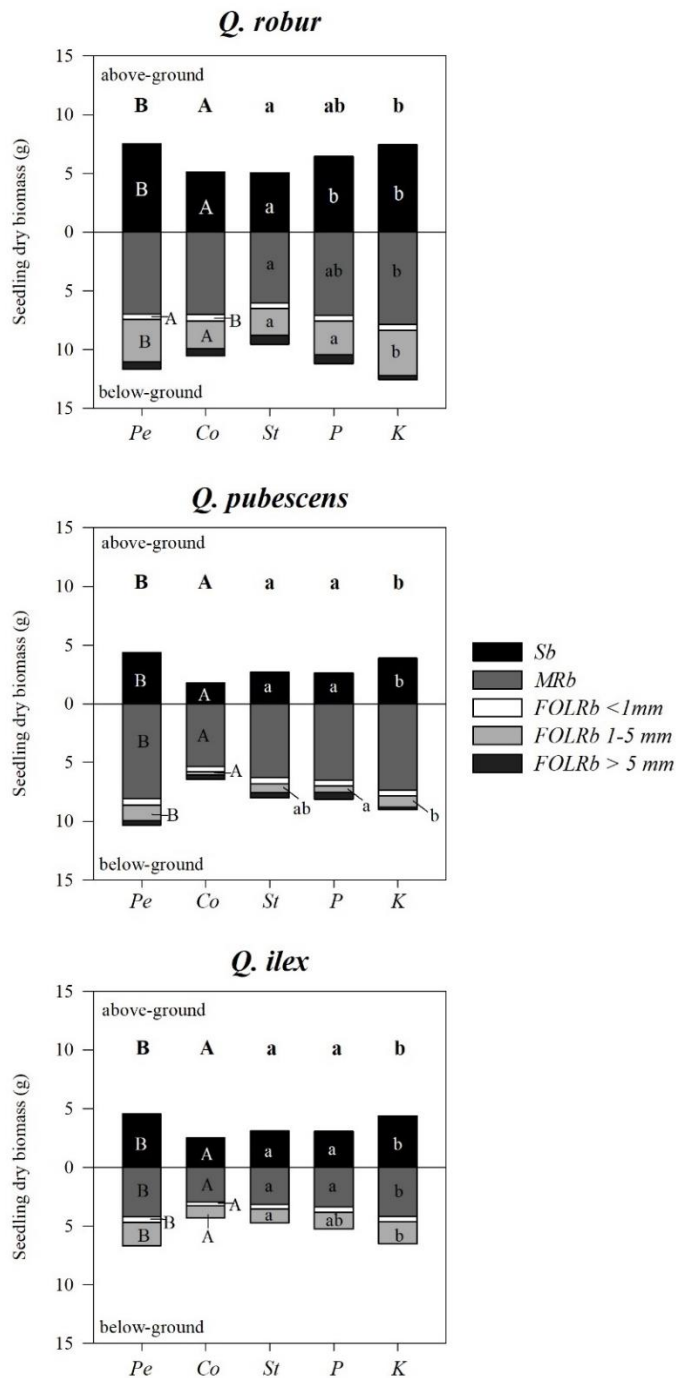
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<i>Q. robur</i>	<i>S</i>	<i>F</i>	<i>Sx</i> <i>F</i>
<i>Sb</i>	<0.0001	<0.0001	0.6355
<i>MRb</i>	0.9205	0.0610	0.7401
<i>FOLRb</i> <1	0.0212	0.5548	0.9441
<i>FOLRb</i> 1-5	<0.0001	<0.0001	0.1665
<i>FOLRb</i> >5	0.8056	0.2704	0.6460
<i>Rb</i>	0.0454	0.0001	0.7101
<i>Sb</i> + <i>Rb</i>	0.0001	<0.0001	0.9119
<i>Q. pubescens</i>			
<i>Sb</i>	<0.0001	0.0002	0.2060
<i>MRb</i>	<0.0001	0.1275	0.2324
<i>FOLRb</i> <1	0.0081	0.1637	0.0127
<i>FOLRb</i> 1-5	<0.0001	0.0075	0.1767
<i>FOLRb</i> >5	0.8859	0.3703	0.4351
<i>Rb</i>	<0.0001	0.3417	0.4148
<i>Sb</i> + <i>Rb</i>	<0.0001	0.0438	0.4563
<i>Q. ilex</i>			
<i>Sb</i>	<0.0001	<0.0001	0.1204
<i>MRb</i>	<0.0001	0.0005	0.0230
<i>FOLRb</i> <1	0.0059	0.5604	0.0674

<i>FOLRb</i> 1-5	<0.0001	0.0013	0.1812
<i>FOLRb</i> >5	-	-	-
<i>Rb</i>	<0.0001	0.0001	0.0389
<i>Sb</i> + <i>Rb</i>	<0.0001	<0.0001	0.0424

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Figure 2. Multifactorial ANOVA and Tukey post hoc test results ($p \leq 0.05$) for seedling dry biomass (g) allocation at the end of the growing season ($N_{obs}=20$ per stocktype per species). Source of variation substrate (S), fertilization (F). *Sb*: shoot-system dry biomass; *MRb*: main root dry biomass; *FOLRb*: dry biomass of First Order Lateral Root split by diameter class (<1 mm, 1-5 mm, >5 mm). Capital letters indicate homogenous groups for substrate, while lowercase letters indicate homogenous groups for fertilization; letters in the upper part indicate groups for total biomass. Pe= Peat; Co=Coir; St=Standard fertilization; K=K-enriched fertilization; P=P-enriched fertilization.



282

283 3.3 N, P, K concentration in seedlings and substrate at the end of cultivation

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285 In *Q. robur*, N concentration was not affected by treatments excluding fertilization on root-
 286 system (*St* and *P>K*), while both substrate (*Pe>Co*) and fertilization (*P* and *K>St*) affected P
 287 concentration in seedling parts (excluding substrate on shoot; Table 3, Figure 3). *Pe-P* had higher P
 288 concentration than other stocktypes in leaves and in shoot (Figure S2). Both *S* and *F* did not influence
 289 K concentration. In *Q. pubescens*, N concentration in any tissue was not influenced by either treatment
 290 (Table 3, Figure 3); P concentration was affected by both substrate (*Pe>Co*) and fertilization (*P* and
 291 *K>St*; excluding substrate on shoot-system Table 3, Figure 3). *Pe-P* had generally higher P content
 292 than other stocktypes in leaves, shoot and roots (Figure S2). Differences in K concentration were
 293 found between substrates in leaves (*Pe>Co*). In *Q. ilex*, both substrate (*Co>Pe*) and fertilization (*St>K*)
 294 influenced plant N concentration in all tissues (Table 3, Figure 3). Substrate affected leaf P
 295 concentration (*Co>Pe*) and fertilization affected root-system concentration (*P>St*), K concentration
 296 was influenced by substrate in roots (*Co>Pe*), and by fertilization in shoot-system (Table 3, Figure 3).

297

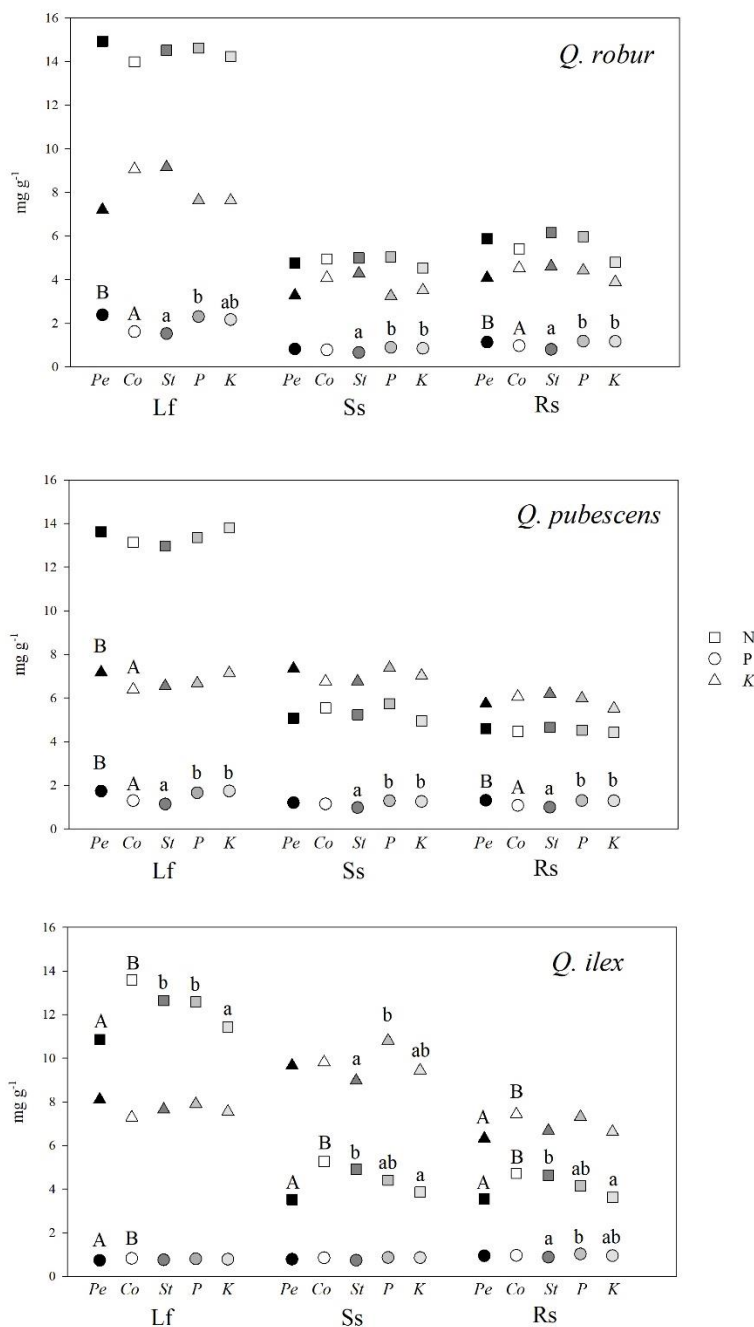
298 **Table 3.** *p* values of multifactorial ANOVA ($p \leq 0.05$ in bold) for and for macro-element concentration (N, P, K) in
 299 leaves, shoot-system and root-system at the end of the growing season (N=6 couples per stocktype). Source of
 300 variation: substrate (*S*), fertilization (*F*), and their interaction (*SxF*). For chlorophyll content (SPAD) and Fv/Fm,
 301 and PI_{ABS} we reported *p*-values calculated from data of each data collection (June, July, and August).

		<i>Q. robur</i>			<i>Q. pubescens</i>			<i>Q. ilex</i>		
		<i>S</i>	<i>F</i>	<i>SxF</i>	<i>S</i>	<i>F</i>	<i>SxF</i>	<i>S</i>	<i>F</i>	<i>SxF</i>
N (mg/g)	Leaves	0.1398	0.8712	0.3971	0.2220	0.2340	0.2340	<0.0001	0.0067	0.3531
	Shoot	0.3956	0.1357	0.1760	0.0922	0.0681	0.0681	<0.0001	0.0027	0.1779
	Root	0.0711	0.0002	0.9724	0.5779	0.6927	0.6927	<0.0001	0.0017	0.2128
P (mg/g)	Leaves	0.0036	0.0317	0.4204	0.0002	0.0001	0.0001	0.0020	0.3830	0.4956
	Shoot	0.4027	0.0013	0.0439	0.3254	0.0001	0.0001	0.1794	0.0923	0.9658
	Root	0.0088	<0.0001	0.0208	0.0034	0.0024	0.0024	0.7227	0.0381	0.5865
K (mg/g)	Leaves	0.1448	0.5164	0.1323	0.0469	0.4150	0.4150	0.0751	0.7980	0.2119
	Shoot	0.4515	0.7074	0.1073	0.2170	0.5737	0.5737	0.8051	0.0423	0.6514
	Root	0.4930	0.6484	0.1861	0.4115	0.3866	0.3866	0.0188	0.3926	0.1645

302

303 **Figure 3.** Macro-element concentration (mg g⁻¹ for N, P, K) in leaves (Lf), shoot (stem, Ss) and root-system (Rs)
 304 analyzed for substrate and fertilization (mean ± SD). Source of variation substrate (*S*), fertilization (*F*), and their
 305 interaction (*SxF*). Capital letters and lowercase letters indicate homogenous groups for *S* and *F*, respectively. *Pe*=
 306 Peat; *Co*=Coir; *St*=Standard fertilization; *K*=K-enriched fertilization; *P*=P-enriched fertilization.

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310 Nitrate N concentration of the growing media with *Q. robur* was affected by substrate and
 311 fertilization ($Pe > Co$; $P > K$), and only by fertilization for ammonium N ($P > K$, Table 4). In *Q. pubescens*,
 312 fertilization affected P concentration ($P > K$), and both nitric and ammonium N ($P > K$). In *Q. ilex*, nitrate
 313 N was affected by fertilization ($P > K$) and ammonium N by both factors ($Co > Pe$; $P > K$), and P
 314 concentration resulted not affected by substrate and fertilization, while both affected K concentration
 315 ($Co > Pe$; $K > P$ and St). No interaction among factors occurred.

316

317 **Table 4.** Multifactorial ANOVA and Tukey post-hoc test results ($p \leq 0.05$ in bold) for (mean \pm SD, N = 6) macro-
 318 element concentration (N, P, K) in meq/l contained in the growing medium at the end of the season (N=12 per
 319 combination). Source of variation substrate (S), fertilization (F), and their interaction (SxF). Lowercase letters
 320 indicate homogenous groups. Pe = Peat; Co =Coir; St =Standard fertilization; K =K-enriched fertilization; P =P-
 321 enriched fertilization.

<i>Q. robur</i>	<i>S</i>	<i>F</i>	<i>SxF</i>	<i>Pe</i>	<i>Co</i>	<i>St</i>	<i>P</i>	<i>K</i>
Nitrate N	0.0160	0.0004	0.5459	1,32 ± 0.49 B	0.86 ± 0.66 A	1.11 ± 0.36 ab	1.65 ± 0.47 b	0.52 ± 0.39 a
Ammonium N	0.9211	0.0029	0.2031	1,02 ± 0.34	1.01 ± 0.54	0.92 ± 0.26 ab	1.44 ± 0.37 b	0.69 ± 0.29 a
P	0.2960	0.9878	0.3515	0,26 ± 0.08	0.22 ± 0.06	0.24 ± 0.06	0.23 ± 0.05	0.24 ± 0.11
K	0.3836	0.0821	0.9781	0,89 ± 0.24	0.79 ± 0.25	0.66 ± 0.13	0.88 ± 0.26	0.98 ± 0.23
<i>Q. pubescens</i>								
Nitrate N	0.0612	0.0020	0.3632	1,22 ± 0.70 B	0.83 ± 0.48 A	0.84 ± 0.42 a	1.64 ± 0.54 b	0.61 ± 0.35 a
Ammonium N	0.7522	0.0036	0.6327	0,96 ± 0.55	0.91 ± 0.37	0.81 ± 0.26 ab	1.39 ± 0.38 b	0.60 ± 0.28 a
P	0.9340	0.0222	0.6351	0,19 ± 0.07	0.19 ± 0.04	0.18 ± 0.04 ab	0.24 ± 0.05 b	0.15 ± 0.04 a
K	0.5296	0.1437	0.5819	0,73 ± 0.25	0.87 ± 0.64	0.50 ± 0.13	1.07 ± 0.70	0.83 ± 0.26
<i>Q. ilex</i>								
Nitrate N	0.7839	0.0212	0.4642	0,65 ± 0.51	0.71 ± 0.51	0.77 ± 0.35 ab	1.02 ± 0.55 b	0.25 ± 0.20 a
Ammonium N	0.0299	0.0260	0.3370	0,44 ± 0.36 A	0.83 ± 0.46 B	0.65 ± 0.39 ab	0.93 ± 0.53 b	0.32 ± 0.14 a
P	0.0824	0.3603	0.8507	0,15 ± 0.06	0.21 ± 0.08	0.17 ± 0.05	0.22 ± 0.11	0.16 ± 0.05
K	0.0295	0.0005	0.7127	0,68 ± 0.34 A	0.90 ± 0.28 B	0.59 ± 0.23 a	0.65 ± 0.25 a	1.14 ± 0.14 b

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323

324 **4. Discussion**

325

326 Our study highlighted effects of substrate on seedling growth that began quite early (2 months
327 after sowing in *Q. robur* and *Q. pubescens*; 3 months in *Q. ilex*) and persisted through cultivation. The
328 absence of any fertilization effect on early growth phases was expected in all species: dependence of
329 *Quercus* seedlings on acorn nutrients decreases as seedlings develop [7,71] and plants start to rely on
330 soil/substrate properties. Thus, the development of the first growth flush in many *Quercus* species is
331 to a higher extent related to acorn size and nutrients in the seed [72,73], rather than to the quality of
332 the growing medium or soil to [7,71]. Our results are in line with those of Villar-Salvador et al. [71]
333 in *Q. ilex*: fertilization effect was significant just at the end of the second growth flush and beyond.
334 The delayed effect observed in *Q. ilex* in comparison with the other species can be explained by a later
335 emergence of holm oak seedlings, which could have altered the occurrence of the substrate effect.
336 From late spring to the end of cultivation, seedlings of all species grown in peat were taller than those
337 grown in coir. However, height of seedlings in coir were at least equal to that of comparable forest
338 nursery production systems of *Quercus* species [27,74–76]. Our results confirmed those of Rose and
339 Haase [61] with Douglas fir, in which seedlings grew more with peat than coir. Tsakalidimi and
340 Ganatsas [11], using similar containers as in this study, also reported that *Q. ilex* seedlings grown in
341 a peat-perlite mixture (3:1) were taller than in peat-coir mixture (1:1). Radjagukguk et al. [62] showed
342 high mortality and delayed growth for two *Eucalyptus* species grown in coir, while Offord et al. [77]
343 did not observe differences between plants grown in peat with sand and perlite, and in coir in the
344 same mixture in several species, including *Eucalyptus melliodora*. The reduced growth rate observed
345 in seedlings grown in coir could be due to the lower CEC than in peat, which reduces nutrients
346 availability for seedlings. This could explain the smaller size of the vast majority of the studied
347 morphological traits in all coir stocktypes in all species regardless of fertilization. Additionally,
348 according to Handreck [78], there is a greater immobilization of soluble nitrogen in coir than peat..
349 Grantzau [79] similarly highlighted that the microbial activity in coir caused nitrogen immobilization,
350 suggesting the need to add extra N fertilization during cultivation [78,79]; however, in our study we
351 did not find reductions in tissue concentration of seedlings raised with coir, suggesting an absence of
352 nitrogen deficiency. The common mixtures and fertilization protocols used by nursery companies in
353 horti-flori-fructiculture sector have been optimized [80] and plants in coir grow equally as peat
354 mixtures [29,77,81,82]. Other studies aim at excessive salinity, which often characterizes coir [9,61],
355 to explain the negatively affected root development and physiology [83], and thereby altered growth.
356 However, in our study, coir EC was in line with the values reported in literature [84,85], with similar
357 or lower values than in peat.

358 According to our results, morphological traits variability was explained more by substrate than
359 by fertilization. Fertilization effects on growth occurred later than those of growing medium, in mid-

360 summer for *Q. pubescens* and *Q. ilex* and near the end of the growing season in *Q. robur*. In all species
361 and in both growing media, the K-enriched fertilization improved the growth rate during the
362 growing season and promoted taller plants. Apart from N fertilization, scarce literature is available
363 on the effect of the other macro-elements on growth of forest species in the nursery [7]. K is
364 recognized as a key element of many metabolic processes [86], some of which are related to plant
365 stress responses. Direct connections of K with seedling growth are less investigated, but in a meta-
366 analysis of forest species (including plants at seedling stage), Tripler et al. [87] highlighted that plant
367 growth responded positively to an increase in K availability. Studies on tropical forest species
368 highlighted a direct effect of K fertilization in promoting plant growth with a higher shoot/root ratio
369 [88,89]. Similar to height responses, seedlings in all species grown in peat and K-enriched fertilization
370 allocated more resources to above-ground dry biomass (leaves and shoot system). Similar results for
371 substrate effects were obtained by Rose and Haase [61] in Douglas-fir. Soto Castelblanco [90], who
372 studied seedlings of *Q. ilex* and three *Pinus* species grown in peat and in coir, found higher, despite
373 non-significant, values of shoot-system biomass in peat. Coir K content is usually higher than that in
374 peat [31,61]. However, in our study, despite the tested rates of K are high compared to literature on oaks
375 [91–93] we observed an outstanding effect of such fertilization on plant growth regardless
376 species or substrate, suggesting K deficiency status. K enriched fertilization included an additional
377 amount of P as compared to Standard fertilizer, which could have been helpful in sustaining seedling
378 growth. However, fertilization with P was not as effective as K in promoting height and biomass in
379 these studied species, although, generally, it was better than standard fertilization. P plays a key role
380 in plant metabolism, being a critical element for many physiological reactions [94]. In agriculture and
381 forestry, the action of P is linked to several root morphological traits, such as root-system structure,
382 growth and articulation, rather than to above-ground development [49,50,94–96]. Thus, a non-
383 pronounced effect on shoot system growth was expected.

384 In all studied species and treatments, peat and K-enriched fertilization promoted the greatest
385 below-ground development. In all cases, shoot/root ratio was <1, and cultivation in coir reduced this
386 ratio further, particularly for *Q. pubescens*. Other studies on flowering species pointed out that
387 cultivation in coir can promote a proportionally higher root system component [81,97]. The same
388 effect occurred on Douglas fir, with higher root biomass for seedlings in coir and a decreasing trend
389 in S/R ratio from peat, to a mixture of coir and peat, to coir [61]. In contrast, Tsakalimi and Ganastas
390 [11] found a higher S/R ratio in 1-year-old seedlings of *Q. macrolepis* and *Q. ilex* grown in a mixture
391 of peat and coconut fiber than in peat with perlite. A lower shoot to root ratio has been linked to
392 survival under drought condition in holm oak [98], in Mediterranean shrubs [99] and savanna species
393 [100]. On the contrary, Villar-Salvador et al. [40] highlighted that *Q. ilex* seedlings with a higher S/R
394 had lower mortality and larger growth than those with the opposite attributes; however, we found
395 quite similar shoot to root values for holm oak produced in coir to the highest values observed in this
396 study (0.59 vs 0.63, respectively).

397 Peat also promoted main root biomass and favored root fibrosity, especially the two smaller
398 classes of *FOLR* (>1 mm and 1-5 mm). The proportion of *FOLR* on total root biomass was statistically
399 affected by substrate (data not showed) in *Q. robur* and *Q. ilex*; however, the gap between substrates
400 in all species was slight. This result suggests that plants grown in coir, despite a lower absolute root-
401 system biomass, maintained comparable root fibrosity to peat in a proportionally higher below-
402 ground component. This result is inconsistent with Chulaka et al. [101] and Wilson et al. [102] who
403 reported the effects of coir-based substrates on S/R ratio with non-significant results *versus* peat-based
404 substrates and with Colla et al [97], who found a higher S/R ratio in horticultural species grown in
405 peat. Studies on tropical tree species and in alpine and dryland environment [88,89] reported that K
406 promoted shoot biomass and less *FOLR*, resulting in a lower S/R ratio. Our results partially confirmed
407 such observations, as with K enriched fertilization the S/R ratio was higher, but the biomass allocated
408 to *FOLR* was positively influenced by such fertilization. P-enriched fertilization did not promote the
409 growth and articulation of the root-system as expected, even though this study did not compare
410 different levels of P and K, but rather three different fertilizations. Scarce literature has focused on
411 the direct effect of P on detailed morphological below ground traits in *Quercus*. According to Pemán

412 [7], root systems of *Quercus* species can be affected by available P; Sardans et al. [103] found that P
413 fertilization can promote root growth in *Q. ilex* ssp. *ballota* and Villar-Salvador et al. [40] linked P
414 concentration to capacity of regenerating roots in holm oak. Oliet et al. [47] highlighted a clear effect
415 of root P concentration at final nursery sampling and root growth potential (RGP) in *Q. ilex* seedlings.

416 Physiological results are in agreement with morphology, demonstrating an overall better
417 performance of photosynthetic machinery (considering SPAD and F_v/F_m in conjunction) of seedlings
418 in peat and seedlings fertilized with enriched K in most species. Chlorophyll fluorescence, even
419 though measured values show absence of stress, has proven to be a sensitive technique to detect
420 differences among stocktypes, especially those from different fertilization treatments. Results for
421 Chlorophyll content were generally consistent with what was observed in morphology, and peat and
422 K-enriched fertilization promoted higher values in all species. The values of Chlorophyll content
423 observed for deciduous oaks were in line with those in other studies [104,105].

424 We did not find a marked effect of substrate or fertilization on N concentration in plant tissues,
425 and results were quite variable among species. At the end of cultivation, N concentration of *Q. robur*
426 and *Q. pubescens* did not depend on substrate and only sporadically on fertilization, whereas both
427 factors affected *Q. ilex*. Despite a higher N content per seedling than that from other studies on the
428 same species [91,106], N concentration in holm oak roots and shoot-system was lower. In our study,
429 N supply per seedling was higher than the sufficiency level in *Q. ilex* (125 mg in exponential
430 fertilization rate) according to Uscola et al. [91], and higher than 200 mg indicated as an optimum
431 target by the same study. Uscola et al. [91] applied fertilizer via irrigation with an exponential regime,
432 which could contribute to increase uptake efficiency [107]. In *Q. petraea*, a European temperate
433 *Quercus* species, Berger and Glatzer [108] reported 400 mg per seedling as an N amount for luxury
434 consumption; we supplied 27% less N, which was insufficient to affect *Q. robur* tissue N
435 concentration. However, levels of consumption can vary not only in relation to experimental factors,
436 but also to intrinsic ecological differences among species, such that, even among *Quercus*
437 Mediterranean species, the sufficiency levels are quite different [91,93]. This makes speculation on *Q.*
438 *pubescens* difficult, as this issue is not well represented in the literature for this species. Peat as well
439 as P-enriched fertilization were effective in increasing the level of P in all plant tissues in *Q. robur* and
440 *Q. pubescens*, while in *Q. ilex* such fertilization was effective only for P concentration in roots, and in
441 contrast to the other species, holm oak seedlings in coir performed better than in peat. Higher soil P
442 availability [109–113] and root tissue concentration are important to promote root growth capacity
443 after planting. Del Campo et al. [98], in a study designed to define quality standards for *Q. ilex* nursery
444 stock, indicated that values of N and P foliar concentration should be higher than 10 and 0.9 mg g⁻¹,
445 respectively, to improve growth performance after planting. Considering this target, our results for
446 N were higher and better in peat and in P-enriched and Standard fertilization, and for P were about
447 10% lower and higher in peat. No references are available for *Q. robur* and *Q. pubescens*; however, in
448 our study, foliar N and P concentrations in these deciduous species were higher than the standard
449 values for the evergreen *Q. ilex*. In our study, K concentration in plant tissues was generally not
450 affected by both substrate and fertilization in all species suggesting that K-enriched fertilization did
451 not increase K concentration. Considering the higher performance of seedlings grown in K-enriched
452 fertilization regardless species or substrate, this result strengthens the hypothesis that *Quercus* species
453 benefit from high K availability and that the amount provided in Standard and in P-enriched
454 fertilization was deficient. According to [92], K is the most responsive nutrient at deficiency status.
455 The vast majority of literature on fertilization of forest nursery stock has focused on N effects; thus,
456 more investigations on optimal K rates to maximize growth is needed. Del Campo et al. [98]
457 concluded that nursery stock with higher K concentration can exhibit improved establishment
458 success due to K retranslocation. Andivia et al. [114] observed that fertilization with K positively
459 influenced morphological development and NPK content. K effects on field performance on *Pinus*
460 *halepensis* have been observed with conflicting results [115,116]. Del Campo et al. [117] suggested that
461 these multiple responses could be related to the interference of K with other nutrients, particularly
462 nitrogen. The interaction between K fertilizers and different types of substrate requires further
463 investigation.

464

465 **5. Conclusions**

466

467 Peat and coir in combination with different fertilization treatments affected above- and below-
468 ground morphology and, to a lesser extent, physiological traits of *Quercus* seedlings in forest nursery
469 production. The three studied species, despite being ecologically diverse, provided similar results,
470 with peat and K-enriched fertilization resulting in larger seedlings and slightly improved
471 physiological responses. The smaller size of seedlings in coir could be linked to deficiencies in the
472 chemical properties of this material, which should be balanced by fertilization. Nevertheless,
473 seedlings produced in coir in this study were compatible with the size of standard *Quercus* forest
474 stocktypes and with root system development and fibrosity. According to the Target Plant Concept
475 [3,5], functional traits driving planting performance vary according to environmental and operational
476 conditions; and plants with a lower shoot to root ratio may perform better under water stress or in
477 semiarid/arid environments [98,118]. Coir may thus serve as an acceptable material for seedling
478 cultivation in such cases, despite the tendency to produce smaller seedlings. NPK content was slightly
479 influenced by treatments, although P-enriched fertilization in peat was the only combination that
480 promoted a higher amount of this element in tissues at the end of cultivation; the strong effect of K-
481 enriched fertilization on seedling growth and biomass suggested a K deficiency in the other
482 fertilization treatments; however, macro-element content in relation to varying fertilization should
483 be further investigated by comparison of the single element rates.

484

485 **Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Table S1: Macro-
486 elements (NPK) content (per volume and per seedling) in the studied fertilizations; Table S2: Multifactorial
487 ANOVA and Tukey post hoc test results ($p \leq 0.05$ in bold) for seedling morphological traits (mean \pm SD) at the
488 end of the season ($N_{\text{obs}} = 20$ seedlings per stocktype); Table S3: Multifactorial ANOVA and Tukey post-hoc test
489 results ($p \leq 0.05$ in bold) for (mean \pm SD) macro-element concentration (N, P, K) in meq/l contained in the growing
490 medium at the end of the season ($N=3$ couple per combination); Figure S1: Multifactorial ANOVA and Tukey
491 post hoc test results ($p \leq 0.05$) for seedling dry biomass (g) allocation at the end of the growing season ($N_{\text{obs}}=20$
492 per stocktype per species); Figure S2: Macro-element concentration (mg g⁻¹ for N, P, K) in leaves, shoot-system
493 and root-system in stocktypes (mean \pm SD).

494

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811 **Supplementary Material**

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813 **Table S1:** Macro-elements (NPK) content (per volume and per seedling) in the studied fertilizations.

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	Content per volume mg/l			Content per seedling (mg per pot)		
	N	P	K	N	P	K
<i>Standard</i>	450	270	330	292,5	175,5	214,5
<i>P enriched</i>	450	690	330	292,5	448,5	214,5
<i>K enriched</i>	440	440	720	286	286	468

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Table S2: Multifactorial ANOVA and Tukey post hoc test results ($p \leq 0.05$ in bold) for seedling morphological traits (mean \pm SD) at the end of the season ($N_{\text{obs}} = 20$ seedlings per stocktype). Source of variation: substrate (S), fertilization (F), and their interaction (SxF). Lowercase letters indicate homogeneous groups. Variables: *H*: height; *RcD* (root collar diameter in mm); *H/RcD* (seedling taper); *Rv* (root-system volume in cm^3); *Rd* (root-system density= Rb/Rv in g cm^{-3}); *S/R* (shoot-root ratio); *SLA* (specific leaf area, in $\text{cm}^2 \text{g}^{-1}$); *Ln* (number of leaves); *Lb* (leaf dry biomass in g).

<i>Q. robur</i>	<i>Pe-St</i>	<i>Pe-P</i>	<i>Pe-K</i>	<i>Co-St</i>	<i>Co-P</i>	<i>Co-K</i>
<i>H</i>	53.51 \pm 9.94 abc	58.12 \pm 10.91 bc	61.15 \pm 10.67 c	43.09 \pm 10.62 a	44.82 \pm 9.61 a	49.60 \pm 10.08 ab
<i>RcD</i>	10.3 \pm 1.8 ab	11.6 \pm 1.9 b	11.6 \pm 1.9 b	9.9 \pm 1.8 a	10.9 \pm 1.6 ab	11.6 \pm 1.2 b
<i>H/RcD</i>	53.2 \pm 13.8 b	50.7 \pm 8.9 ab	53.8 \pm 11.7 b	43.7 \pm 9.4 a	41.6 \pm 8.5 a	43.3 \pm 9.7 a
<i>Rv</i>	22.6 \pm 6.4 ab	27.0 \pm 8.2 b	34.4 \pm 8.7 c	19.4 \pm 5.2 a	26.9 \pm 7.9 b	27.6 \pm 7.6 b
<i>Rd</i>	0.46 \pm 0.08 ab	0.43 \pm 0.06 ab	0.38 \pm 0.05 a	0.47 \pm 0.12 b	0.41 \pm 0.07 ab	0.44 \pm 0.06 ab
<i>S/R</i>	0.59 \pm 0.15 bc	0.66 \pm 0.08 c	0.70 \pm 0.16 c	0.46 \pm 0.16 a	0.49 \pm 0.13 ab	0.52 \pm 0.16 ab
<i>SLA</i>	155.2 \pm 22.8 ab	145.9 \pm 38.5 ab	167.0 \pm 20.5 b	138.8 \pm 13.4 a	138.6 \pm 24.4 a	148.8 \pm 13.0 ab
<i>Ln</i>	46.2 \pm 12.4 ab	51.1 \pm 25.2 ab	72.5 \pm 30.0 c	37.1 \pm 12.7 a	44.9 \pm 18.2 ab	59.8 \pm 20.7 bc
<i>Lb</i>	3.58 \pm 1.06 ab	4.40 \pm 1.15 b	5.56 \pm 1.50 c	2.75 \pm 1.06 a	3.60 \pm 1.25 ab	3.91 \pm 1.25 b
<i>Q. pubescens</i>	<i>Pe-St</i>	<i>Pe-P</i>	<i>Pe-K</i>	<i>Co-St</i>	<i>Co-P</i>	<i>Co-K</i>
<i>H</i>	34.29 \pm 10.13 bc	33.38 \pm 10.06 bc	42.75 \pm 12.39 c	21.41 \pm 6.78 a	20.67 \pm 8.08 a	25.14 \pm 9.17 ab
<i>RcD</i>	10.1 \pm 1.8 abc	10.3 \pm 2.0 bc	11.6 \pm 1.6 c	9.4 \pm 1.9 ab	8.4 \pm 2.3 a	10.2 \pm 2.2 abc
<i>H/RcD</i>	34.5 \pm 11.0 c	32.8 \pm 9.6 bc	37.1 \pm 10.4 c	23.2 \pm 6.5 a	24.8 \pm 7.5 ab	24.5 \pm 7.8 a
<i>Rv</i>	28.7 \pm 13.5 c	22.7 \pm 8.0 bc	25.9 \pm 8.8 c	13.1 \pm 3.5 a	15.0 \pm 7.4 ab	16.7 \pm 6.5 ab
<i>Rd</i>	0.38 \pm 0.07 a	0.42 \pm 0.06 ab	0.42 \pm 0.06 ab	0.45 \pm 0.07 b	0.45 \pm 0.06 b	0.41 \pm 0.04 ab
<i>S/R</i>	0.37 \pm 0.10 bc	0.42 \pm 0.14 cd	0.51 \pm 0.13 d	0.28 \pm 0.10 ab	0.19 \pm 0.07 a	0.33 \pm 0.13 bc
<i>SLA</i>	102.7 \pm 8.6	105.5 \pm 17.5	105.7 \pm 13.1	100.0 \pm 9.4	102.1 \pm 8.8	99.7 \pm 8.2
<i>Ln</i>	62.8 \pm 34.6 c	51.6 \pm 24.2 bc	67.2 \pm 19.8 c	30.2 \pm 7.5 a	31.8 \pm 8.8 a	42.7 \pm 21.7 ab
<i>Lb</i>	4.41 \pm 1.49 b	3.74 \pm 1.30 b	4.82 \pm 1.69 b	1.88 \pm 0.78 a	1.95 \pm 0.92 a	2.52 \pm 1.39 a
<i>Q. ilex</i>	<i>Pe-St</i>	<i>Pe-P</i>	<i>Pe-K</i>	<i>Co-St</i>	<i>Co-P</i>	<i>Co-K</i>
<i>H</i>	48.36 \pm 10.90 bc	49.90 \pm 12.00 cd	59.93 \pm 13.80 d	33.37 \pm 10.38 a	33.65 \pm 8.95 a	37.87 \pm 11.31 ab
<i>RcD</i>	8.3 \pm 0.8	8.4 \pm 1.4	8.8 \pm 1.0	7.9 \pm 1.7	7.4 \pm 1.6	8.2 \pm 1.5
<i>H/RcD</i>	58.4 \pm 11.8 bc	60.1 \pm 12.9 c	69.6 \pm 18.8 c	41.8 \pm 9.2 a	47.0 \pm 15.9 ab	46.3 \pm 11.0 ab
<i>Rv</i>	12.2 \pm 4.1 ab	15.4 \pm 6.0 bc	17.0 \pm 3.3 c	9.4 \pm 3.8 a	10.3 \pm 3.2 a	12.2 \pm 5.5 ab
<i>Rd</i>	0.46 \pm 0.12	0.41 \pm 0.09	0.49 \pm 0.07	0.45 \pm 0.14	0.42 \pm 0.07	0.43 \pm 0.15
<i>S/R</i>	0.73 \pm 0.23 b	0.71 \pm 0.36 ab	0.70 \pm 0.10 ab	0.62 \pm 0.25 ab	0.51 \pm 0.13 a	0.65 \pm 0.25 ab
<i>SLA</i>	78.0 \pm 7.3 b	70.7 \pm 8.7 a	71.9 \pm 6.3 a	68.1 \pm 3.6 a	70.7 \pm 5.3 a	72.5 \pm 6.6 ab
<i>Ln</i>	46.9 \pm 14.3 bc	44.5 \pm 13.8 b	56.8 \pm 16.6 c	30.3 \pm 13.7 a	27.5 \pm 6.0 a	32.3 \pm 12.1 a
<i>Lb</i>	4.63 \pm 1.12 b	4.92 \pm 1.81 b	6.15 \pm 1.21 c	2.70 \pm 1.26 a	2.83 \pm 1.03 a	3.08 \pm 1.27 a

Figure S1: Multifactorial ANOVA and Tukey post hoc test results ($p \leq 0.05$) for seedling dry biomass (g) allocation at the end of the growing season ($N_{obs}=20$ per stocktype per species). Source of variation substrate (S) fertilization (F) and their interaction (SxF). *Sb*: shoot-system dry biomass; *MRb*: main root dry biomass; *FOLRb*: dry biomass of First Order Lateral Root split by diameter class (<1 mm, 1-5 mm, >5 mm). Capital letters indicate homogenous groups for total biomass while lowercase letters indicate homogenous groups for the remaining variables. *Pe*= Peat; *Co*=Coir; *St*=Standard fertilization; *K*=K-enriched fertilization; *P*=P-enriched fertilization.

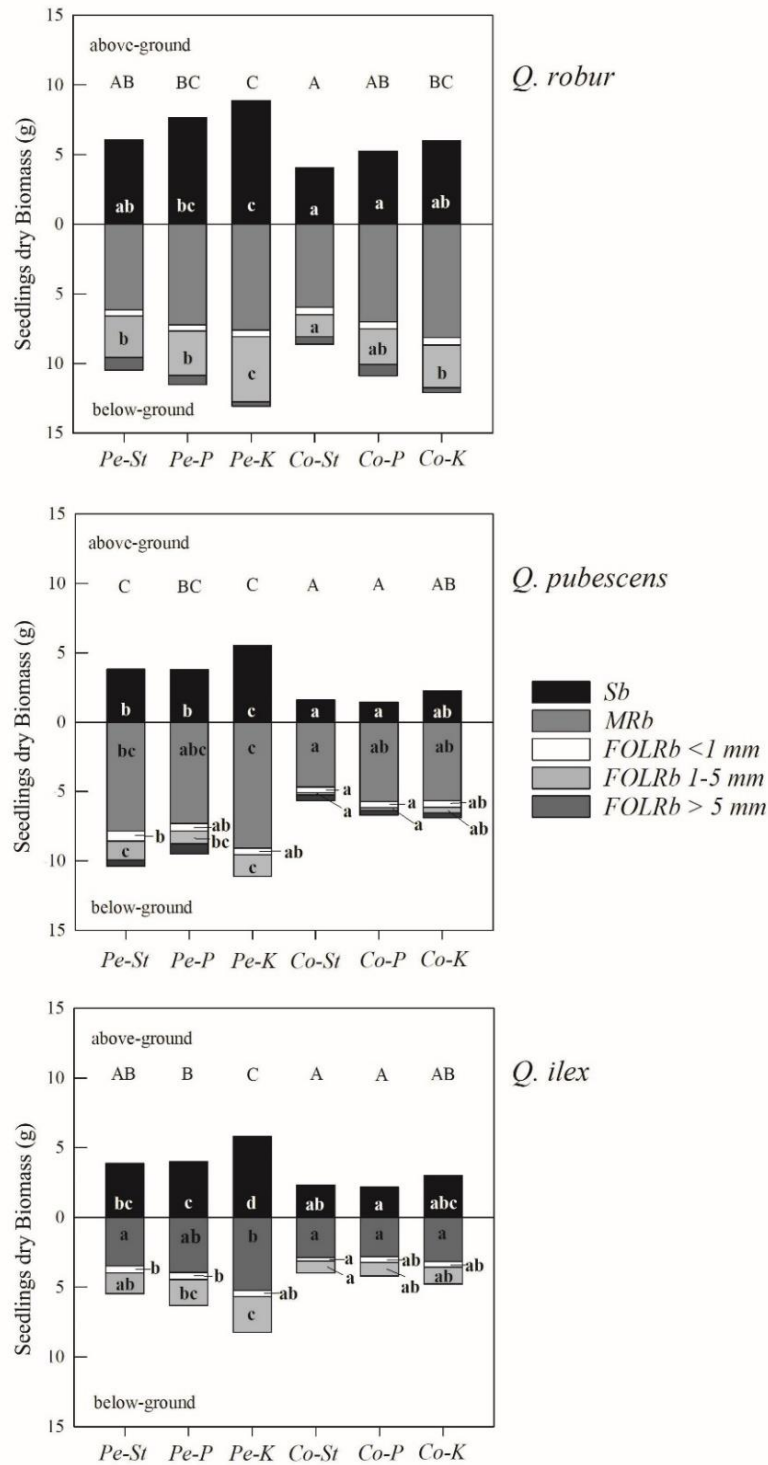


Figure S2: Macro-element concentration (mg g^{-1} for N, P, K) in leaves, shoot-system and root-system in stocktypes (mean \pm SD). Source of variation substrate (S), fertilization (F) and their interaction (SxF). Lowercase letters indicate homogenous groups. *Pe*= Peat; *Co*=Coir; *St*=Standard fertilization; *K*=K-enriched fertilization; *P*=P-enriched fertilization.

