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Coconut coir as a sustainable nursery growing media for seedling production of the ecologically diverse quercus species

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- 2 Article

³ Pure coconut coir as a sustainable nursery growing

4 media for seedling production of ecologically diverse

- 5 Quercus species
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19 Abstract: Peat, a non-sustainable resource, is still predominately used in forest nurseries. Coconut 20 coir may provide an alternative, renewable, and reliable growing media but few studies have 21 evaluated this media type in forest nurseries. We assessed the influence of pure coir in combination 22 with various fertilization regimes on the growth and physiology of three ecologically diverse 23 Quercus species seedlings (Q. robur, Q. pubescens, Q. ilex) during nursery cultivation. Seedlings were 24 grown using peat and pure coir in combination with 3 fertilization treatments (standard, K enriched 25 , P enriched). Data were collected for: 1) growth and physiological traits; 2) detailed above- and 26 below-ground morphological traits by destructive analysis; and, 3) NPK content in leaves, shoot 27 and roots, and in growing media following cultivation.

Peat and coir in combination with the various fertilization treatments affected above- and belowground 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. Penriched 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.

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

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48 1. Introduction

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

125126 2. Materials and Methods

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128 2.1 Nursery stock cultivation

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

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

A total of 6 treatments combinations per species (2 substrates x 3 fertilizations) were included inthe 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.).

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165 2.2 Data collection

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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 Fv/FM (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 x 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 (Ln and Lb), root-collar 183 diameter (RcD) and dry biomass of the shoot (stem) were measured. Root-system was assessed by 184 main root (tap-root) dry biomass (MRb); first order lateral roots (FOLR) dry biomass (FOLRb) 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 (*Rv*, *Rb* and *Rd* resp.). Shoot to root ratio (*S*/*R*), *H*/*RcD*, 187 and Specific Leaf Area (SLA) were also calculated.

- 188
- **189** 2.3 Statistical analysis
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191 A randomized complete block design (3 blocks) was used. Each block included the 6 randomized 192 S x 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, Yijhmn = µ + Blocki 194 + Date_j + Substrate(S)_k + fertilization(F)₁ + SxF interaction_{kl} + 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 (SxF) as source of 198 variation. In case of significant results ($p \le 0.05$), Tukey post hoc test was used for multiple 199 comparisons (α =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 (SxF). To provide 201 information about the traits of the studied stocktypes (combinations SxF), 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

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207 3.1 Emergence, growth and physiological traits during nursery cultivation

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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 that 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.

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Table 1. Multifactorial ANOVA and Tukey post hoc test results ($p \le 0.05$ in bold) for seedling morphological traits (mean ± SD) at the end of the season ($N_{obs} = 20$ seedlings per stocktype per species). Source of variation: substrate (*S*), fertilization (*F*), and their interaction (*SxF*). Capital and lowercase letters indicate homogeneous groups for *S* and *F*, resp. 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 *cm*² *g*⁻

1); *Ln* (number of leaves); *Lb* (leaf dry biomass in *g*).

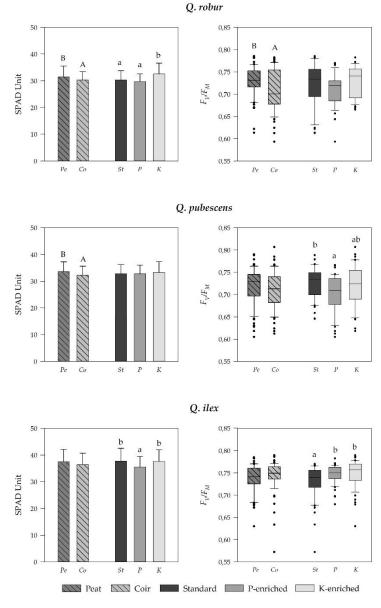
Q. robur	S	F	SxF	Pe	Со	St	Р	K
Н	<0.0001	0.0108	0.8213	57.6 ± 10,8 B	$45.8\pm10.3~{\rm A}$	48.3 ± 11.4 a	51.5 ± 12.2 ab	55.4 ± 11.8 b
RcD	0.2265	0.0006	0.6354	11.2 ± 1.9	10.8 ± 1.7	$10.1 \pm 1.8 \ a$	$11.2 \pm 1.8 \text{ ab}$	$11.6 \pm 1.6 \ \mathbf{b}$
H/RcD	<0.0001	0.4976	0.9521	52.6 ± 11.3 B	$42.9\pm9.1~{\rm A}$	48.4 ± 12.2	46.1 ± 9.8	48.6 ± 11.9
Rv	0.0143	0.0001	0.1355	$28.0\pm9.2~\textbf{B}$	$24.6\pm7.8\;\mathbf{A}$	21.0 ± 6.0 a	$26.9\pm8.0~\textbf{b}$	$31.0 \pm 8.8 \ \mathbf{b}$
Rd	0.3264	0.0040	0.0711	0.43 ± 0.07	0.44 ± 0.09	$0.47\pm0.10~\mathbf{b}$	0.42 ± 0.06 ab	0.41 ± 0.06 a
S/R	<0.0001	0.0419	0.6985	$0.65\pm0.14~\mathbf{B}$	$0.49\pm0.15~{\rm A}$	0.53 ± 0.17 a	$0.57\pm0.14~\mathbf{ab}$	$0.61\pm0.18~\mathbf{b}$
SLA	0.0016	0.0120	0.5480	156.1 ± 29.2 B	142.1 ± 18.1 A	147.0 ± 20.2 ab	142.2 ± 32.0 a	157.9 ± 19.3 b
Ln	0.0156	<0.0001	0.7843	$56.6\pm26.0~\mathbf{B}$	$47.2\pm19.7~{\rm A}$	41.6 ± 13.2 a	48.0 ± 22.0 ab	66.1 ± 26.2 b
Lb	<0.0001	<0.0001	0.2137	4.5 ± 1.5 B	3.4 ± 1.3 A	3.2 ± 1.1 a	$4.0 \pm 1.3 \ \mathbf{b}$	$4.7\pm1.6~\mathbf{b}$
Q. pubescens	S	F	SxF	Ре	Со	St	Р	Κ
Н	<0.0001	0.0028	0.4337	36.8 ± 11.5 B	$22.4\pm8.2~{\rm A}$	$27.9 \pm 10.7 \text{ ab}$	27.0 ± 11.1 a	33.9 ± 14.0 b
RcD	0.0003	0.0024	0.4202	$10.7\pm1.9\;\mathbf{B}$	$9.4 \pm 2.2 \ \mathbf{A}$	9.7 ± 1.9 ab	9.4 ± 2.3 a	$10.9\pm2.0~\mathbf{b}$
H/RcD	<0.0001	0.5218	0.5139	$34.8 \pm 10.3 \text{ B}$	$24.2\pm7.2~{\rm A}$	28.9 ± 10.6	28.9 ± 9.5	30.8 ± 11.1
Rv	<0.0001	0.3988	0.0951	$25.8\pm10.5~\textbf{B}$	$14.9\pm6.1~{\rm A}$	20.9 ± 12.6	19.1 ± 8.6	21.3 ± 9.0
Rd	0.0101	0.2732	0.0215	$0.41\pm0.07\;{\rm A}$	$0.44\pm0.06~\mathbf{B}$	0.42 ± 0.08	0.43 ± 0.06	0.42 ± 0.05
S/R	<0.0001	0.0001	0.0725	$0.43 \pm 0.13 \text{ B}$	$0.27\pm0.11~{\rm A}$	0.33 ± 0.11 a	0.31 ± 0.15 a	$0.42\pm0.16~\mathbf{b}$
SLA	0.0600	0.6396	0.7851	104.6 ± 13.4	100.6 ± 8.7	101.4 ± 9.0	103.8 ± 13.9	102.7 ± 11.2
Ln	<0.0001	0.0232	0.4068	$60.5\pm27.2~\mathbf{B}$	$34.9 \pm 15.1 \; \mathbf{A}$	$46.5\pm29.7~\text{ab}$	41.7 ± 20.5 a	$54.9 \pm 24.0 \ \mathbf{b}$
Lb	<0.0001	0.0192	0.4390	$4.3 \pm 1.5 \text{ B}$	$2.1 \pm 1.1 \text{ A}$	3.1 ± 1.7 ab	2.9 ± 1.4 a	3.7 ± 1.9 b
Q. ilex	S	F	SxF	Ре	Со	St	Р	Κ
Н	<0.0001	0.0032	0.3332	52.7 ± 13.1 B	$35.0\pm10.3~{\rm A}$	40.9 ± 13.0 a	41.8 ± 13.3 ab	48.9 ± 16.7 b
RcD	0.0114	0.1610	0.5929	$8.5\pm1.1~{\rm B}$	7.8 ± 1.6 A	8.1 ± 1.3	7.9 ± 1.5	8.5 ± 1.3
H/RcD	<0.0001	0.0386	0.2352	62.7 ± 15.4 B	$45.1\pm12.3~{\rm A}$	50.1 ± 13.4 a	53.6 ± 15.7 ab	58.0 ± 19.2 b
Rv	< 0.0001	0.0009	0.4449	14.9 ± 5.0 B	10.7 ± 4.3 A	10.8 ± 4.2 a	12.9 ± 5.4 ab	14.6 ± 5.1 b

Rd	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
S/R	0.0062	0.3922	0.3637	$0.71\pm0.25~\textbf{B}$	$0.59\pm0.22~{\rm A}$	0.68 ± 0.24	0.61 ± 0.29	0.67 ± 0.19
SLA	0.0104	0.2676	0.0005	73.6 ± 8.0 B	$70.5\pm5.5~{\rm A}$	73.2 ± 7.6	70.7 ± 7.1	72.2 ± 6.4
Ln	<0.0001	0.0140	0.3323	49.4 ± 15.6 B	$30.0\pm11.1~{\rm A}$	38.6 ± 16.2 ab	36.0 ± 13.6 a	$44.5\pm19.0~\mathbf{b}$
Lb	<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

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

231

232Figure 1. Multifactorial ANOVA and Tukey post-hoc test results of Fv/F_M values (box whisker plot) and233SPAD units (mean and SD) analyzed for substrate and fertilization (Nobs=81 for substrate; Nobs=54 for234fertilization) in August. Capital and lowercase letters indicate homogenous groups for substrate and235fertilization, respectively. Pe= Peat; Co=Coir; St=Standard fertilization; K=K-enriched fertilization; P=P-enriched236fertilization



239 *3.2 Morphological traits*

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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

268Table 2. p values from multifactorial ANOVA test results: (p < 0.05 in bold) for seedlings biomass allocation269(Nobs =20 per stocktype per species). Sources of variation: substrate (S), fertilization (F), and their interaction270(SxF). Sb: shoot-system biomass; MRb: main root biomass; FOLRb: First Order Lateral Root biomass split by271diameter class (<1 mm, 1-5 mm, >5 mm); Rb: total root-system biomass; Sb + Rb: total seedling biomass.

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

< 0.0001	0.0013	0.1812
-	-	-
<0.0001	0.0001	0.0389
< 0.0001	<0.0001	0.0424
	- <0.0001	<0.0001 0.0013 <0.0001 0.0001 <0.0001 <0.0001

274

Figure 2. Multifactorial ANOVA and Tukey post hoc test results (p≤0.05) for seedling dry biomass (g) allocation at the end of the growing season (Nobs=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.

281

Q. robur 15 above-ground B ab b A a 10 Seedling dry biomass (g) 5 R b 0 5 B 10 b below-ground 15 St P K Pe Co Q. pubescens 15 above-ground B b A a 10 a Seedling dry biomass (g) 5 Sb MRb B 0 FOLRb <1mm FOLRb 1-5 mm 5 FOLRb > 5 mm10 below-ground 15 P Ċo St K Pe Q. ilex 15 above-ground B b 10 A a a Seedling dry biomass (g) 5 0 5

K

P

10

15

below-ground

Co

St

Pe

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

284

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 ($p \le 0.05$ 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,

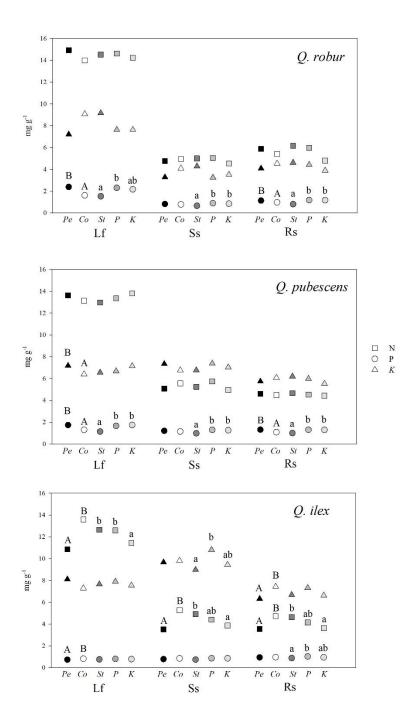
301 and PIABS we reported p-values calculated from data of each data collection (June, July, and August).

		Q. robur			Q	Q. pubescens			Q. ilex		
		S	F	SxF	S	F	SxF	S	F	SxF	
N	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	
(mg/g)	Root	0.0711	0.0002	0.9724	0.5779	0.6927	0.6927	<0.0001	0.0017	0.2128	
Р	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	
(mg/g)	Root	0.0088	<0.0001	0.0208	0.0034	0.0024	0.0024	0.7227	0.0381	0.5865	
V	Leaves	0.1448	0.5164	0.1323	0.0469	0.4150	0.4150	0.0751	0.7980	0.2119	
K	Shoot	0.4515	0.7074	0.1073	0.2170	0.5737	0.5737	0.8051	0.0423	0.6514	
(mg/g)	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 \pm 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.



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

316

Table 4. Multifactorial ANOVA and Tukey post-hoc test results ($p \le 0.05$ in bold) for (mean \pm SD, N = 6) macro-

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.

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Q. robur	S	F	SxF	Pe	Со	St	Р	Κ
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	$1,02 \pm 0.34$	1.01 ± 0.54	0.92 ± 0.26 ab	1.44 ± 0.37 b	0.69 ± 0.29 a
Р	0.2960	0.9878	0.3515	$0,26 \pm 0.08$	0.22 ± 0.06	0.24 ± 0.06	0.23 ±0.05	0.24 ± 0.11
К	0.3836	0.0821	0.9781	$0,89 \pm 0.24$	0.79 ± 0.25	0.66 ± 0.13	0.88 ± 0.26	0.98 ± 0.23
Q. pubescens								
Nitrate N	0.0612	0.0020	0.3632	1,22 ± 0.70 B	$0.83\pm0.48~{\rm A}$	0.84 ± 0.42 a	1.64 ± 0.54 b	0.61 ± 0.35 a
Ammonium N	0.7522	0.0036	0.6327	$0,96 \pm 0.55$	0.91 ± 0.37	0.81 ± 0.26 ab	$1.39 \pm 0.38 \ \mathbf{b}$	0.60 ± 0.28 a
Р	0.9340	0.0222	0.6351	$0,19 \pm 0.07$	0.19 ± 0.04	$0.18\pm0.04~\mathbf{ab}$	$0.24\pm0.05~\mathbf{b}$	0.15 ± 0.04 a
К	0.5296	0.1437	0.5819	$0,73 \pm 0.25$	0.87 ± 0.64	0.50 ± 0.13	1.07 ± 0.70	0.83 ± 0.26
Q. ilex								
Nitrate N	0.7839	0.0212	0.4642	$0,65 \pm 0.51$	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	$0,44 \pm 0.36$ A	$0.83 \pm 0.46 \text{ B}$	0.65 ± 0.39 ab	0.93 ± 0.53 b	0.32 ± 0.14 a
Р	0.0824	0.3603	0.8507	$0,15 \pm 0.06$	0.21 ± 0.08	0.17 ± 0.05	0.22 ± 0.11	0.16 ± 0.05
К	0.0295	0.0005	0.7127	$0,68 \pm 0.34$ A	0.90 ± 0.28 B	0.59 ± 0.23 a	0.65 ± 0.25 a	$1.14 \pm 0.14 \text{ b}$

322 323

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. Tsakaldimi 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.

According to our results, morphological traits variability was explained more by substrate than by fertilization. Fertilization effects on growth occurred later than those of growing medium, in mid360 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 375 oaks [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, Tsakaldimi 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 [7], root systems of *Quercus* species can be affected by available P; Sardans et al. [103] found that P
fertilization can promote root growth in *Q. ilex* ssp. *ballota* and Villar-Salvador et al.[40] linked P
concentration to capacity of regenerating roots in holm oak. Oliet et al. [47] highlighted a clear effect

of root P concentration at final nursery sampling and root growth potential (RGP) in *Q. ilex* seedlings. Physiological results are in agreement with morphology, demonstrating an overall better performance of photosynthetic machinery (considering SPAD and *Fv/F_M* in conjunction) of seedlings in peat and seedlings fertilized with enriched K in most species. Chlorophyll fluorescence, even though measured values show absence of stress, has proven to be a sensitive technique to detect differences among stocktypes, especially those from different fertilization treatments. Results for Chlorophyll content were generally consistent with what was observed in morphology, and peat and K-enriched fertilization promoted higher values in all species. The values of Chlorophyll content

K-enriched fertilization promoted higher values in all species. The values of Chlorophyll content
observed for deciduous oaks were in line with those in other studies [104,105].
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 Qurecus 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.

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≤0.05 in bold) for seedling morphological traits (mean ± SD) at the 488 end of the season (Nobs = 20 seedlings per stocktype); Table S3: Multifactorial ANOVA and Tukey post-hoc test 489 results (p≤0.05 in bold) for (mean ± 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≤0.05) for seedling dry biomass (g) allocation at the end of the growing season (Nobs=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 ± SD).

494

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- 514 References
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810

811 Supplementary Material

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

	Con	tent per vo mg/l	lume	Content per seedling (mg per pot)			
	Ν	P	К	N	Р	K	
Standard	450	270	330	292,5	175,5	214,5	
P enriched	450	690	330	292,5	448,5	214,5	
K enriched	440	440	720	286	286	468	

Table S2: Multifactorial ANOVA and Tukey post hoc test results ($p \le 0.05$ in bold) for seedling morphological traits (mean \pm SD) at the end of the season (N_{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 *cm*² *g*⁻¹); *Ln* (number of leaves); *Lb* (leaf dry biomass in *g*).

Q. robur	Pe-St	Pe-P	Pe-K	Co-St	Со-Р	Со-К
Н	53.51 ± 9.94 abc	58.12 ± 10.91 bc	61.15 ± 10.67 c	43.09 ± 10.62 a	44.82 ± 9.61 a	49.60 ± 10.08 ab
RcD	10.3 ± 1.8 ab	$11.6 \pm 1.9 \ \mathbf{b}$	$11.6\pm1.9~\mathbf{b}$	9.9 ± 1.8 a	$10.9\pm1.6~\mathbf{ab}$	11.6 ± 1.2 b
H/RcD	$53.2 \pm 13.8 \ \mathbf{b}$	50.7 ± 8.9 ab	53.8 ± 11.7 b	43.7 ± 9.4 a	41.6 ± 8.5 a	43.3 ± 9.7 a
Rv	22.6 ± 6.4 ab	$27.0\pm8.2~\mathbf{b}$	34.4 ± 8.7 c	19.4 ± 5.2 a	26.9 ± 7.9 b	27.6 ± 7.6 b
Rd	0.46 ± 0.08 ab	$0.43 \pm 0.06 \ ab$	$0.38 \pm 0.05 \text{ a}$	$0.47\pm0.12~\mathbf{b}$	$0.41\pm0.07~{\rm ab}$	0.44 ± 0.06 ab
S/R	$0.59\pm0.15\mathbf{bc}$	0.66 ± 0.08 c	0.70 ± 0.16 c	0.46 ± 0.16 a	0.49 ± 0.13 ab	0.52 ± 0.16 ab
SLA	155.2 ± 22.8 ab	$145.9 \pm 38.5 \text{ ab}$	167.0 ± 20.5 b	138.8 ± 13.4 a	138.6 ± 24.4 a	$148.8 \pm 13.0 \text{ ab}$
Ln	46.2 ± 12.4 ab	51.1 ± 25.2 ab	72.5 ± 30.0 c	37.1 ± 12.7 a	$44.9 \pm 18.2 \text{ ab}$	59.8 ± 20.7 bc
Lb	3.58 ± 1.06 ab	$4.40 \pm 1.15 \ \mathbf{b}$	5.56 ± 1.50 c	2.75 ± 1.06 a	3.60 ± 1.25 ab	3.91 ± 1.25 b
Q. pubescens	Pe-St	Pe-P	Pe-K	Co-St	Со-Р	Со-К
Н	34.29 ± 10.13 bc	33.38 ± 10.06 bc	42.75 ± 12.39 c	21.41 ± 6.78 a	20.67 ± 8.08 a	25.14 ± 9.17 ab
RcD	10.1 ± 1.8 abc	10.3± 2.0 bc	11.6± 1.6 c	9.4± 1.9 ab	8.4± 2.3 a	10.2± 2.2 abc
H/RcD	34.5 ± 11.0 c	32.8 ± 9.6 bc	37.1 ± 10.4 c	23.2 ± 6.5 a	24.8 ± 7.5 ab	24.5 ± 7.8 a
Rv	28.7 ± 13.5 c	22.7 ± 8.0 bc	25.9 ± 8.8 c	13.1 ± 3.5 a	15.0 ± 7.4 ab	16.7 ± 6.5 ab
Rd	0.38 ± 0.07 a	$0.42 \pm 0.06 \ ab$	0.42 ± 0.06 ab	$0.45\pm0.07~\mathbf{b}$	$0.45\pm0.06~\mathbf{b}$	$0.41 \pm 0.04 \text{ ab}$
S/R	$0.37\pm0.10~{\rm bc}$	$0.42 \pm 0.14 \text{ cd}$	$0.51 \pm 0.13 \ d$	0.28 ± 0.10 ab	0.19 ± 0.07 a	0.33 ± 0.13 bc
SLA	102.7 ± 8.6	105.5 ± 17.5	105.7 ± 13.1	100.0 ± 9.4	102.1 ± 8.8	99.7 ± 8.2
Ln	62.8 ± 34.6 c	51.6 ± 24.2 bc	67.2 ± 19.8 c	30.2 ± 7.5 a	31.8 ± 8.8 a	42.7 ± 21.7 ab
Lb	4.41 ± 1.49 b	3.74 ± 1.30 b	$4.82 \pm 1.69 \ \mathbf{b}$	1.88 ± 0.78 a	1.95 ± 0.92 a	2.52 ± 1.39 a
Q. ilex	Pe-St	Pe-P	Pe-K	Co-St	Со-Р	Со-К
Н	48.36 ± 10.90 bc	49.90 ± 12.00 cd	59.93 ± 13.80 d	33.37 ± 10.38 a	33.65 ± 8.95 a	37.87 ± 11.31 ab
RcD	8.3 ± 0.8	8.4 ± 1.4	8.8 ± 1.0	7.9 ± 1.7	7.4 ± 1.6	8.2 ± 1.5
H/RcD	$58.4\pm11.8~\mathbf{bc}$	60.1 ± 12.9 c	69.6 ± 18.8 c	41.8 ± 9.2 a	$47.0\pm15.9~\mathbf{ab}$	$46.3 \pm 11.0 \text{ ab}$
Rv	$12.2 \pm 4.1 \text{ ab}$	15.4 ± 6.0 bc	17.0 ± 3.3 c	9.4 ± 3.8 a	10.3 ± 3.2 a	12.2 ± 5.5 ab
Rd	0.46 ± 0.12	0.41 ± 0.09	0.49 ± 0.07	0.45 ± 0.14	0.42 ± 0.07	0.43 ± 0.15
S/R	$0.73 \pm 0.23 \ \mathbf{b}$	0.71 ± 0.36 ab	$0.70\pm0.10~\mathbf{ab}$	0.62 ± 0.25 ab	0.51 ± 0.13 a	$0.65 \pm 0.25 \text{ ab}$
SLA	78.0 ± 7.3 b	70.7 ± 8.7 a	71.9 ± 6.3 a	68.1 ± 3.6 a	70.7 ± 5.3 a	72.5 ± 6.6 ab
Ln	$46.9 \pm 14.3 \mathbf{bc}$	44.5 ± 13.8 b	56.8 ± 16.6 c	30.3 ± 13.7 a	27.5 ± 6.0 a	32.3 ± 12.1 a
Lb	4.63 ± 1.12 b	4.92 ± 1.81 b	6.15 ± 1.21 c	2.70 ± 1.26 a	2.83 ± 1.03 a	3.08 ± 1.27 a

Figure S1: Multifactorial ANOVA and Tukey post hoc test results ($p\leq0.05$) 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.

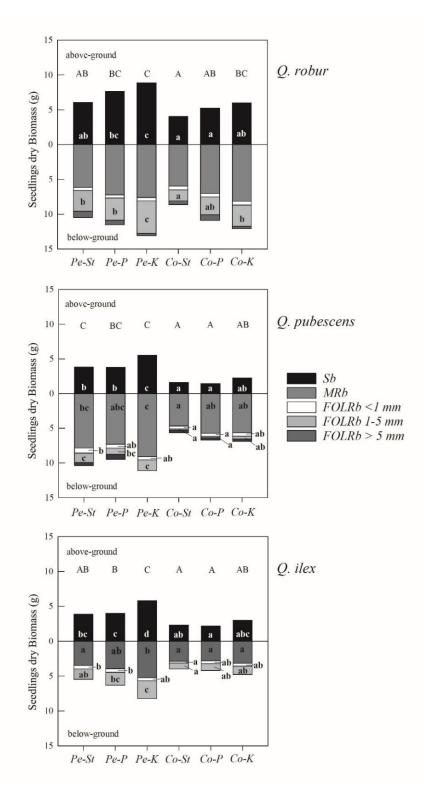


Figure S2: Macro-element concentration (mg g⁻¹ for N. P. K) in leaves, shoot-system and root-system in stocktypes (mean \pm SD). Source of variation substrate (*S*), fertilization (*F*) and their interaction (*SxF*). Lowercase letters indicate homogenous groups. *Pe*= Peat; *Co*=Coir; *St*=Standard fertilization;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

