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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.

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].

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

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

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

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

2. Materials and Methods

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 cm^3 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/m^3, 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/m^3, porosity 92.5%. The week before sowing, the cavities were uniformly filled with growing media to 0.5 cm beneath the top.

Standard fertilization (*St*), a CRC fertilizer widely used in Italy to commercially grow oak seedling nursery stock, was considered as control, and it was compared to two alternative commercial fertilization formulas: enriched in potassium (*K*) or phosphorus (*P*). *St* formula was Osmocote Exact Standard 12-14 months NPK 15-9-11 ^{plus} micronutrients at 3 $kg{\cdot}m^{-3}$. P enriched substrate contained an addition of 19% P_2O_5 fast release (single superphosphate, SSP, 2 $kg{\cdot}m^{-3}$) to control, while K enriched substrate was obtained by 4 $kg{\cdot}m^{-3}$ of Osmocote Exact Standard High K 12-14 months NPK 11-11-18 plus micronutrients. N, P, and K amount per seedling is showed in Table S1.

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

Seed was obtained from the National Center for Biodiversity (Italy); seed provenances were the following for *Q. robur*, *Q. pubescens*, and *Q. ilex*, respectively: Bosco Fontana (43°55'31.4"N, 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). The 1,000-seed weight and germination of the three seed lots (according to ISTA procedures) were 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 October 2016 to April 2017, the acorns were stored at 3 ± 0.5 °C in moist sand to simulate normal overwintering and to prevent acorn germination before the experiment started. In March 2017, the seeds were moved outside to stimulate germination, and then the pre-germinated acorns (0.5 cm maximum radicle length) were sown in multi-pots placed under a tunnel protected by transparent plastic film that was removed at mid-May. Seedlings were irrigated daily by sprinklers following seedling evapotranspirative demands (i.e. spring: 5 $l \cdot m^{-2}$ in 6 min; summer and autumn: 40 l/m^2 and 20 l/m^2, resp. in 24 min). Environmental conditions (air temperature and humidity) were monitored by a weather station (inside the tunnel) and substrate moisture was assessed once a week (6 pots per stocktype, Soil moisture meter PCESMM1, PCE Instr. Corp.).

2.2 Data collection

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

Macro-element (N, P, K) were assessed in October (before leaf abscission) on 12 seedlings per stocktype (*S* x *F*) per species, merging 2 plants of the same multi-pot, by Nutrilab (Universidad Rey Juan Carlos, Madrid, Spain). Briefly, analysis of leaf, stem and root N and P concentration of these samples was done by the standard Kjeldahl method, while K concentration was determined using a perchloric acid extraction. Substrate analysis at the end of the nursery cultivation was performed by Denetra snc (Pescia, Italy) on 9 seedlings per stocktype (*S* x *F*) per species. Concurrently, seedling morphological traits were assessed, using 20 destructively sampled seedlings per treatment combination (360 in total). Height (*H*), number and dry biomass of leaves (*Ln* and *Lb*), root-collar diameter (*RcD*) and dry biomass of the shoot (stem) were measured. Root-system was assessed by main root (tap-root) dry biomass (*MRb*); first order lateral roots (*FOLR*) dry biomass (*FOLRb*) for three diameter classes (<1 mm, 1-5 mm, >5 mm) measured at junction with the tap root, root-system volume (by immersion) and dry weight and density (*Rv*, *Rb* and *Rd* resp.). Shoot to root ratio (*S*/*R*), *H*/*RcD*, and Specific Leaf Area (*SLA)* were also calculated.

2.3 Statistical analysis

A randomized complete block design (3 blocks) was used. Each block included the 6 randomized *S x F* combinations per species; each combination comprised 48 sowed cavities (over 4 multi-pots), for a total of 2592 sowed containers. In a preliminary multifactorial ANOVA (model, $Y_{ijlmn} = \mu + \text{Block}_i + \text{Date}_j + \text{Substrate}(S)_k + \text{fertilization}(F)_l + S\text{x}F\ \text{interaction}_{kl} + \text{error}_{ijklm}$), the block effect was not significant for any morphological variables, highlighting homogeneous growing conditions. Multifactorial ANOVA was performed, separately for each species to avoid complex higher-level interactions, considering substrate (*S*), fertilization (*F*), and their interactions (*SxF*) as source of variation. In case of significant results ($p \leq 0.05$), Tukey post hoc test was used for multiple comparisons ($\alpha = 0.05$) to highlight homogenous groups within species. In this study, we present results related to substrate (*S*), fertilization (*F*), and their significant interactions (*S*x*F*). To provide information about the traits of the studied stocktypes (combinations *S*x*F*), Tukey test results among combinations are shown in supplementary material. StatSoft Statistica 11 (Tulsa, Oklahoma, USA) was used to process all data.

3. Results

3.1 Emergence, growth and physiological traits during nursery cultivation

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

Table 1. Multifactorial ANOVA and Tukey post hoc test results ($p \leq 0.05$ 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*).

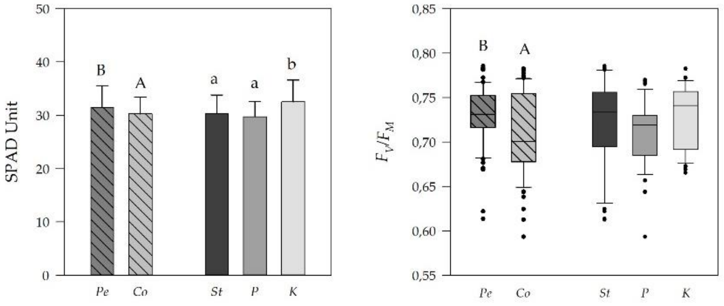
Q. robur	*S*	*F*	*SxF*	*Pe*	*Co*	*St*	*P*	*K*
H	**<0.0001**	**0.0108**	0.8213	57.6 ± 10,8 **B**	45.8 ± 10.3 **A**	48.3 ± 11.4 **a**	51.5 ± 12.2 **ab**	55.4 ± 11.8 **b**
RcD	0.2265	**0.0006**	0.6354	11.2 ± 1.9	10.8 ± 1.7	10.1 ± 1.8 **a**	11.2 ± 1.8 **ab**	11.6 ± 1.6 **b**
H/RcD	**<0.0001**	0.4976	0.9521	52.6 ± 11.3 **B**	42.9 ± 9.1 **A**	48.4 ± 12.2	46.1 ± 9.8	48.6 ± 11.9
Rv	**0.0143**	**0.0001**	0.1355	28.0 ± 9.2 **B**	24.6 ± 7.8 **A**	21.0 ± 6.0 **a**	26.9 ± 8.0 **b**	31.0 ± 8.8 **b**
Rd	0.3264	**0.0040**	0.0711	0.43 ± 0.07	0.44 ± 0.09	0.47 ± 0.10 **b**	0.42 ± 0.06 **ab**	0.41 ± 0.06 **a**
S/R	**<0.0001**	**0.0419**	0.6985	0.65 ± 0.14 **B**	0.49 ± 0.15 **A**	0.53 ± 0.17 **a**	0.57 ± 0.14 **ab**	0.61 ± 0.18 **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 ± 26.0 **B**	47.2 ± 19.7 **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 ± 1.3 **b**	4.7 ± 1.6 **b**
Q. pubescens	*S*	*F*	*SxF*	*Pe*	*Co*	*St*	*P*	*K*
H	**<0.0001**	**0.0028**	0.4337	36.8 ± 11.5 **B**	22.4 ± 8.2 **A**	27.9 ± 10.7 **ab**	27.0 ± 11.1 **a**	33.9 ± 14.0 **b**
RcD	**0.0003**	**0.0024**	0.4202	10.7 ± 1.9 **B**	9.4 ± 2.2 **A**	9.7 ± 1.9 **ab**	9.4 ± 2.3 **a**	10.9 ± 2.0 **b**
H/RcD	**<0.0001**	0.5218	0.5139	34.8 ± 10.3 **B**	24.2 ± 7.2 **A**	28.9 ± 10.6	28.9 ± 9.5	30.8 ± 11.1
Rv	**<0.0001**	0.3988	0.0951	25.8 ± 10.5 **B**	14.9 ± 6.1 **A**	20.9 ± 12.6	19.1 ± 8.6	21.3 ± 9.0
Rd	**0.0101**	0.2732	**0.0215**	0.41 ± 0.07 **A**	0.44 ± 0.06 **B**	0.42 ± 0.08	0.43 ± 0.06	0.42 ± 0.05
S/R	**<0.0001**	**0.0001**	0.0725	0.43 ± 0.13 **B**	0.27 ± 0.11 **A**	0.33 ± 0.11 **a**	0.31 ± 0.15 **a**	0.42 ± 0.16 **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 ± 27.2 **B**	34.9 ± 15.1 **A**	46.5 ± 29.7 **ab**	41.7 ± 20.5 **a**	54.9 ± 24.0 **b**
Lb	**<0.0001**	**0.0192**	0.4390	4.3 ± 1.5 **B**	2.1 ± 1.1 **A**	3.1 ± 1.7 **ab**	2.9 ± 1.4 **a**	3.7 ± 1.9 **b**
Q. ilex	*S*	*F*	*SxF*	*Pe*	*Co*	*St*	*P*	*K*
H	**<0.0001**	**0.0032**	0.3332	52.7 ± 13.1 **B**	35.0 ± 10.3 **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 ± 1.1 **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 ± 12.3 **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 ± 0.25 **B**	0.59 ± 0.22 **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 ± 5.5 **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 ± 11.1 **A**	38.6 ± 16.2 **ab**	36.0 ± 13.6 **a**	44.5 ± 19.0 **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**

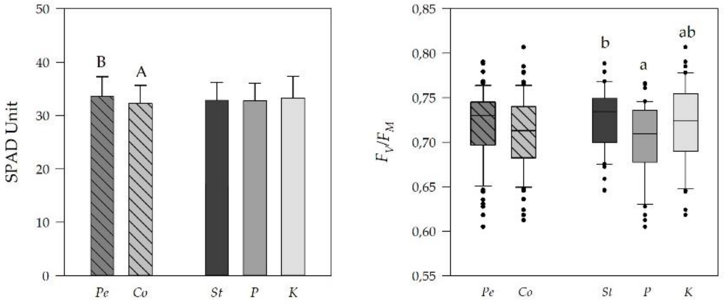
In general, in all species, in August (Figure 1), both substrate (*Pe*>*Co*) and fertilization (*K* generally higher) affected ChlF content, generally with no interactions (data not shown). In *Q. robur* and *Q. pubescens*, seedlings in peat showed higher values of F_V/F_M than in coir (significant in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

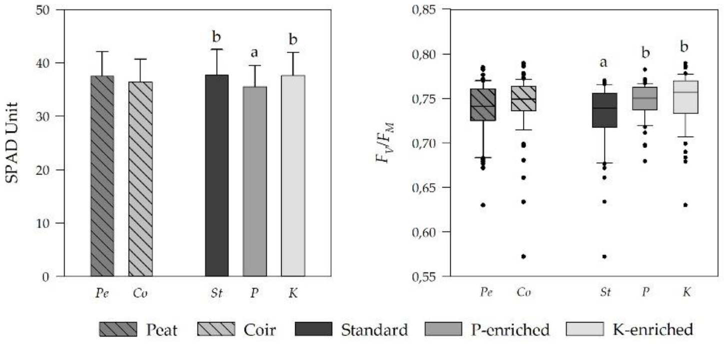
Q. robur



Q. pubescens



Q. ilex



3.2 Morphological traits

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

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

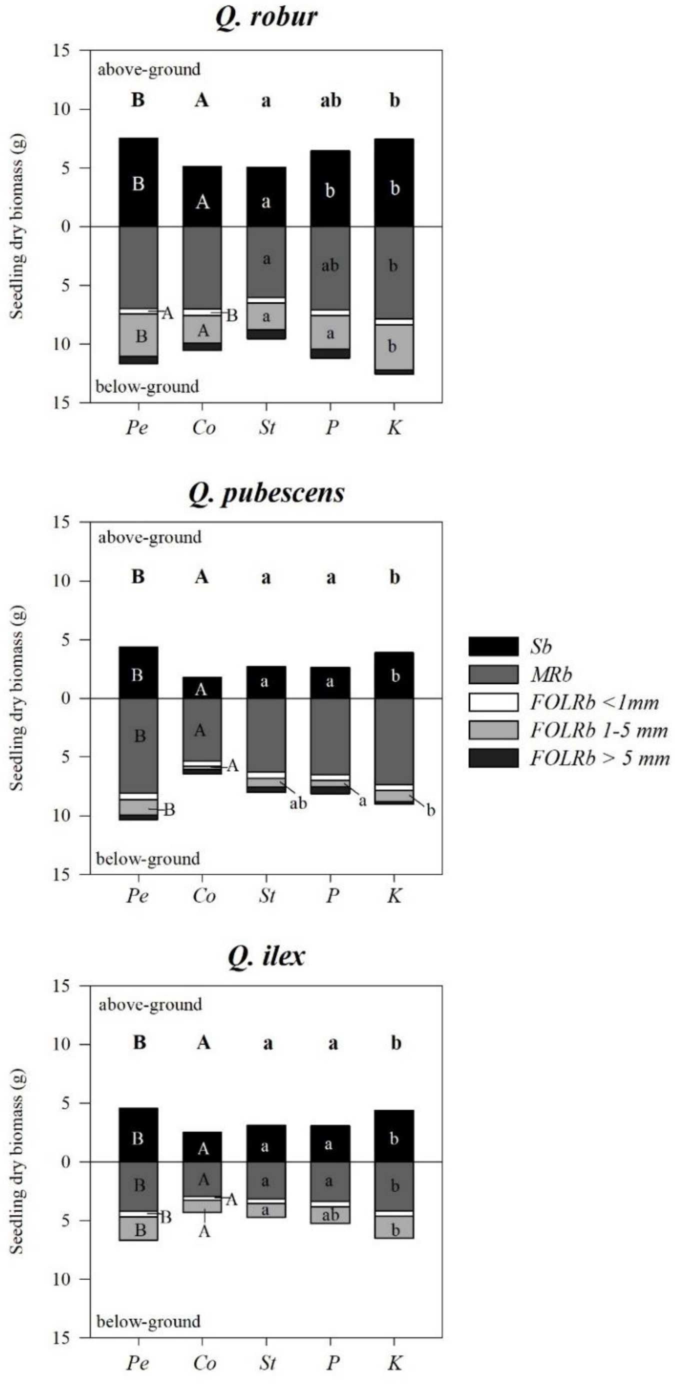
As per previous species, in the case of *Q. ilex,* substrate affected the majority of the analysed variables (with a *Pe*>*Co* pattern), and generally, when fertilization was significant, seedlings grown in K-enriched fertilizer had higher values (Tables 1 and 2, Figure 2). Total biomass was affected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

Table 2. *p* values from multifactorial ANOVA test results: ($p < 0.05$ in bold) for seedlings biomass allocation (N_{obs} =20 per stocktype per species). Sources of variation: substrate (*S*), fertilization (*F*), and their interaction (*SxF*). *Sb*: shoot-system biomass; *MRb*: main root biomass; *FOLRb*: First Order Lateral Root biomass split by diameter 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

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

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.



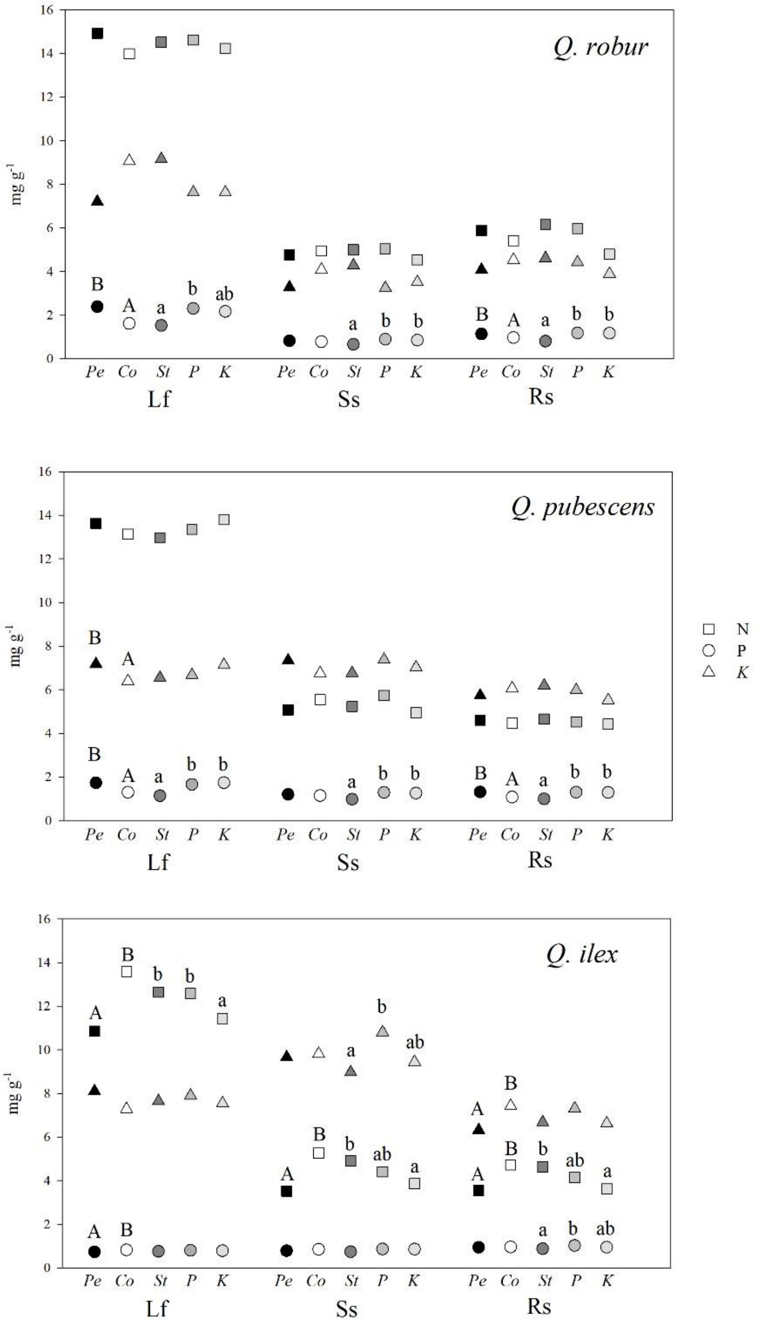
3.3 N, P, K concentration in seedlings and substrate at the end of cultivation

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

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

			Q. robur			***Q. pubescens***			***Q. ilex***	
		S	*F*	*SxF*	*S*	*F*	*SxF*	*S*	*F*	*SxF*
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

Figure 3. Macro-element concentration (mg g^{-1} for N, P, K) in leaves (Lf), shoot (stem, Ss) and root-system (Rs) analyzed for substrate and fertilization (mean ± SD). Source of variation substrate (*S*), fertilization (*F*), and their interaction (*SxF*). Capital letters and lowercase letters indicate homogenous groups for *S* and *F*, respectively. *Pe*= 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.

Table 4. Multifactorial ANOVA and Tukey post-hoc test results (p≤0.05 in bold) for (mean ± 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 combination). 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.

Q. robur	*S*	*F*	*SxF*	*Pe*	*Co*	*St*	*P*	*K*
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
Q. pubescens								
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
Q. ilex								
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**

4. Discussion

Our study highlighted effects of substrate on seedling growth that began quite early (2 months after sowing in *Q. robur* and *Q. pubescens*; 3 months in *Q. ilex*) and persisted through cultivation. The absence of any fertilization effect on early growth phases was expected in all species: dependence of *Quercus* seedlings on acorn nutrients decreases as seedlings develop [7,71] and plants start to rely on soil/substrate properties. Thus, the development of the first growth flush in many *Quercus* species is to a higher extent related to acorn size and nutrients in the seed [72,73], rather than to the quality of the growing medium or soil to [7,71]. Our results are in line with those of Villar-Salvador et al. [71] in *Q. ilex*: fertilization effect was significant just at the end of the second growth flush and beyond. The delayed effect observed in *Q. ilex* in comparison with the other species can be explained by a later emergence of holm oak seedlings, which could have altered the occurrence of the substrate effect. From late spring to the end of cultivation, seedlings of all species grown in peat were taller than those grown in coir. However, height of seedlings in coir were at least equal to that of comparable forest nursery production systems of *Quercu*s species [27,74–76]. Our results confirmed those of Rose and Haase [61] with Douglas fir, in which seedlings grew more with peat than coir. Tsakaldimi and Ganatsas [11], using similar containers as in this study, also reported that *Q. ilex* seedlings grown in a peat-perlite mixture (3:1) were taller than in peat-coir mixture (1:1). Radjagukguk et al. [62] showed high mortality and delayed growth for two *Eucalyptus* species grown in coir, while Offord et al. [77] did not observe differences between plants grown in peat with sand and perlite, and in coir in the same mixture in several species, including *Eucalyptus melliodora.* The reduced growth rate observed in seedlings grown in coir could be due to the lower CEC than in peat, which reduces nutrients availability for seedlings. This could explain the smaller size of the vast majority of the studied morphological traits in all coir stocktypes in all species regardless of fertilization. Additionally, according to Handreck [78], there is a greater immobilization of soluble nitrogen in coir than peat.. Grantzau [79] similarly highlighted that the microbial activity in coir caused nitrogen immobilization, suggesting the need to add extra N fertilization during cultivation [78,79]; however, in our study we did not find reductions in tissue concentration of seedlings raised with coir, suggesting an absence of nitrogen deficiency. The common mixtures and fertilization protocols used by nursery companies in horti-flori-fructiculture sector have been optimized [80] and plants in coir grow equally as peat mixtures [29,77,81,82]. Other studies aim at excessive salinity, which often characterizes coir [9,61], to explain the negatively affected root development and physiology [83], and thereby altered growth. However, in our study, coir EC was in line with the values reported in literature [84,85], with similar 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 midsummer for *Q. pubescens* and Q. *ilex* and near the end of the growing season in *Q. robur*. In all species and in both growing media, the K-enriched fertilization improved the growth rate during the growing season and promoted taller plants. Apart from N fertilization, scarce literature is available on the effect of the other macro-elements on growth of forest species in the nursery [7]. K is recognized as a key element of many metabolic processes [86], some of which are related to plant stress responses. Direct connections of K with seedling growth are less investigated, but in a meta-analysis of forest species (including plants at seedling stage), Tripler et al. [87] highlighted that plant growth responded positively to an increase in K availability. Studies on tropical forest species highlighted a direct effect of K fertilization in promoting plant growth with a higher shoot/root ratio [88,89]. Similar to height responses, seedlings in all species grown in peat and K-enriched fertilization allocated more resources to above-ground dry biomass (leaves and shoot system). Similar results for substrate effects were obtained by Rose and Haase [61] in Douglas-fir. Soto Castelblanco [90], who studied seedlings of *Q. ilex* and three *Pinus* species grown in peat and in coir, found higher, despite non-significant, values of shoot-system biomass in peat. Coir K content is usually higher than that in peat [31,61]. However, in our study, despite the tested rates of K are high compared to literature on oaks [91–93] we observed an outstanding effect of such fertilization on plant growth regardless species or substrate, suggesting K deficiency status. K enriched fertilization included an additional amount of P as compared to Standard fertilizer, which could have been helpful in sustaining seedling growth. However, fertilization with P was not as effective as K in promoting height and biomass in these studied species, although, generally, it was better than standard fertilization. P plays a key role in plant metabolism, being a critical element for many physiological reactions [94]. In agriculture and forestry, the action of P is linked to several root morphological traits, such as root-system structure, growth and articulation, rather than to above-ground development [49,50,94–96]. Thus, a non-pronounced effect on shoot system growth was expected.

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

Peat also promoted main root biomass and favored root fibrosity, especially the two smaller classes of *FOLR* (>1 mm and 1-5 mm). The proportion of *FOLR* on total root biomass was statistically affected by substrate (data not showed) in *Q. robur* and *Q. ilex*; however, the gap between substrates in all species was slight. This result suggests that plants grown in coir, despite a lower absolute root-system biomass, maintained comparable root fibrosity to peat in a proportionally higher below-ground component. This result is inconsistent with Chulaka et al. [101] and Wilson et al. [102] who reported the effects of coir-based substrates on S/R ratio with non-significant results *versus* peat-based substrates and with Colla et al [97], who found a higher S/R ratio in horticultural species grown in peat. Studies on tropical tree species and in alpine and dryland environment [88,89] reported that K promoted shoot biomass and less *FOLR*, resulting in a lower S/R ratio. Our results partially confirmed such observations, as with K enriched fertilization the S/R ratio was higher, but the biomass allocated to *FOLR* was positively influenced by such fertilization. P-enriched fertilization did not promote the growth and articulation of the root-system as expected, even though this study did not compare different levels of P and K, but rather three different fertilizations. Scarce literature has focused on 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 F_V/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 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, and results were quite variable among species. At the end of cultivation, N concentration of *Q. robur* and *Q. pubescens* did not depend on substrate and only sporadically on fertilization, whereas both factors affected *Q. ilex*. Despite a higher N content per seedling than that from other studies on the same species [91,106], N concentration in holm oak roots and shoot-system was lower. In our study, N supply per seedling was higher than the sufficiency level in *Q. ilex* (125 mg in exponential fertilization rate) according to Uscola et al. [91], and higher than 200 mg indicated as an optimum target by the same study. Uscola et al. [91] applied fertilizer via irrigation with an exponential regime, which could contribute to increase uptake efficiency [107]. In *Q. petraea*, a European temperate *Qurecus* species, Berger and Glatzer [108] reported 400 mg per seedling as an N amount for luxury consumption; we supplied 27% less N, which was insufficient to affect *Q. robur* tissue N concentration. However, levels of consumption can vary not only in relation to experimental factors, but also to intrinsic ecological differences among species, such that, even among *Quercus* Mediterranean species, the sufficiency levels are quite different [91,93]. This makes speculation on *Q. pubescens* difficult, as this issue is not well represented in the literature for this species. Peat as well as P-enriched fertilization were effective in increasing the level of P in all plant tissues in *Q. robur* and *Q. pubescens*, while in *Q. ilex* such fertilization was effective only for P concentration in roots, and in contrast to the other species, holm oak seedlings in coir performed better than in peat. Higher soil P availability [109–113] and root tissue concentration are important to promote root growth capacity after planting. Del Campo et al. [98], in a study designed to define quality standards for *Q. ilex* nursery stock, indicated that values of N and P foliar concentration should be higher than 10 and 0.9 mg g^{-1}, respectively, to improve growth performance after planting. Considering this target, our results for N were higher and better in peat and in P-enriched and Standard fertilization, and for P were about 10% lower and higher in peat. No references are available for *Q. robur* and *Q. pubescens*; however, in our study, foliar N and P concentrations in these deciduous species were higher than the standard values for the evergreen Q. *ilex*. In our study, K concentration in plant tissues was generally not affected by both substrate and fertilization in all species suggesting that K-enriched fertilization did not increase K concentration. Considering the higher performance of seedlings grown in K-enriched fertilization regardless species or substrate, this result strengthens the hypothesis that *Quercus* species benefit from high K availability and that the amount provided in Standard and in P-enriched fertilization was deficient. According to [92], K is the most responsive nutrient at deficiency status. The vast majority of literature on fertilization of forest nursery stock has focused on N effects; thus, more investigations on optimal K rates to maximize growth is needed. Del Campo et al. [98] concluded that nursery stock with higher K concentration can exhibit improved establishment success due to K retranslocation. Andivia et al. [114] observed that fertilization with K positively influenced morphological development and NPK content. K effects on field performance on *Pinus halepensis* have been observed with conflicting results [115,116]. Del Campo et al. [117] suggested that these multiple responses could be related to the interference of K with other nutrients, particularly nitrogen. The interaction between K fertilizers and different types of substrate requires further investigation.

5. Conclusions

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

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Macro-elements (NPK) content (per volume and per seedling) in the studied fertilizations; Table S2: Multifactorial ANOVA and Tukey post hoc test results ($p \leq 0.05$ in bold) for seedling morphological traits (mean ± SD) at the end of the season (Nobs = 20 seedlings per stocktype); Table S3: Multifactorial ANOVA and Tukey post-hoc test results ($p \leq 0.05$ in bold) for (mean ± SD) macro-element concentration (N. P. K) in meq/l contained in the growing medium at the end of the season (N=3 couple per combination); 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); Figure S2: Macro-element concentration (mg g^{-1} for N. P. K) in leaves, shoot-system and root-system in stocktypes (mean ± SD).

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Supplementary Material

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

	Content per volume mg/l			**Content per seedling (mg per pot)**		
	N	**P**	**K**	**N**	**P**	**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 ($p \leq 0.05$ in bold) for seedling morphological traits (mean ± 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*	*Co-P*	*Co-K*
H	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 ± 1.9 **b**	11.6 ± 1.9 **b**	9.9 ± 1.8 **a**	10.9 ± 1.6 **ab**	11.6 ± 1.2 **b**
H/RcD	53.2 ± 13.8 **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 ± 8.2 **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 ± 0.06 **ab**	0.38 ± 0.05 **a**	0.47 ± 0.12 **b**	0.41 ± 0.07 **ab**	0.44 ± 0.06 **ab**
S/R	0.59 ± 0.15 **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 ± 38.5 **ab**	167.0 ± 20.5 **b**	138.8 ± 13.4 **a**	138.6 ± 24.4 **a**	148.8 ± 13.0 **ab**
Ln	46.2 ± 12.4 **ab**	51.1 ± 25.2 **ab**	72.5 ± 30.0 **c**	37.1 ± 12.7 **a**	44.9 ± 18.2 **ab**	59.8 ± 20.7 **bc**
Lb	3.58 ± 1.06 **ab**	4.40 ± 1.15 **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*	*Co-P*	*Co-K*
H	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 ± 0.06 **ab**	0.42 ± 0.06 **ab**	0.45 ± 0.07 **b**	0.45 ± 0.06 **b**	0.41 ± 0.04 **ab**
S/R	0.37 ± 0.10 **bc**	0.42 ± 0.14 **cd**	0.51 ± 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 ± 1.69 **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*	*Co-P*	*Co-K*
H	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 ± 11.8 **bc**	60.1 ± 12.9 **c**	69.6 ± 18.8 **c**	41.8 ± 9.2 **a**	47.0 ± 15.9 **ab**	46.3 ± 11.0 **ab**
Rv	12.2 ± 4.1 **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 ± 0.23 **b**	0.71 ± 0.36 **ab**	0.70 ± 0.10 **ab**	0.62 ± 0.25 **ab**	0.51 ± 0.13 **a**	0.65 ± 0.25 **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 ± 14.3 **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 ($p \leq 0.05$) 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.

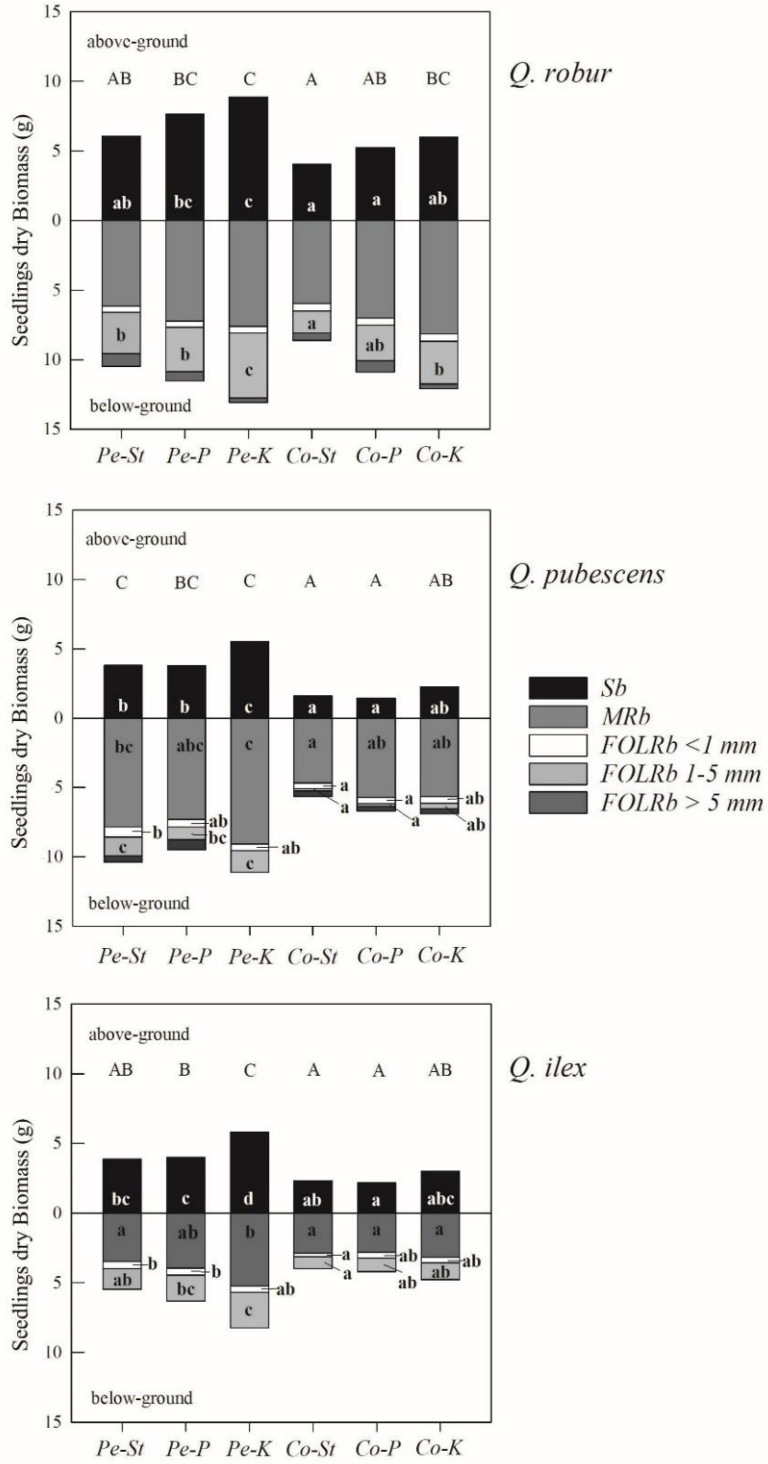

Figure S2: Macro-element concentration (mg g^{-1} for N. P. K) in leaves, shoot-system and root-system in stocktypes (mean ± SD). Source of variation substrate (*S*), fertilization (*F*) and their interaction (*SxF*). Lowercase letters indicate homogenous groups. *Pe*= Peat; *Co*=Coir; *St*=Standard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