Use of raw and acidified biochars as constituents of growth media for forest seedling production

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Abstract In plant nurseries devoted to the propagation of shrubs and trees for landscaping, gardening or forestry it is first concern to produce robust seedlings which resist the stress of transplanting to soil. The selection of appropriate growth media is crucial. Biochar, the product of pyrolysis of organic matter, has been suggested as a new organic amendment for soil or for soilless growth media. Biochar is usually strongly alkaline. We studied the possibility of acidifying biochar with nitric and phosphoric acids. The effects of raw and acidified biochars in peat-based substrates on rooting and growth of cuttings of *Rosmarinus officinalis* and in sandy soil-based substrates on growth of *Phillyrea angustifolia* seedlings were compared. The physical and chemical characteristics of the growth media, and the growth and nutrient content of seedlings were analysed. Results showed that biochar acidification with nitric and phosphoric acids improves the pH and enriches the biochar with N and P without excessively increasing electrical conductivity. However, a column experiment showed that nitrate was readily leached whilst phosphate was tightly retained by biochar, which questioned the practical availability of these nutrients to plants. The agronomical assays showed that both raw biochar and acidified biochar improved rooting and growth of *Rosmarinus* cuttings. In *Phillyrea*, however, the acidified biochar did not affect plant growth whilst the raw biochar gave satisfactory results both for shoot and root growth. Results led to the conclusion that biochar without further treatment might be successfully used as growth medium constituent, even at large proportions, both in organic and in mineral substrates.

Keywords natural park nursery; nutrient leaching curves; *Phillyrea angustifolia*; plant growth; *Rosmarinus officinalis*; rooting cutting

Introduction

Phillyrea angustifolia L. and *Rosmarinus officinalis* are two abundant species in Mediterranean ecosystems. *P. angustifolia* grows well both in calcareous or siliceous soils (classified as indifferent to carbonate by Gastón et al., 2009) and is highly tolerant to drought (Ogaya et al. 2003) and to salinity (Gucci et al. 1997). *R. officinalis* has a preference for calcareous soils although it can also grow in siliceous soils (non-strict calcicole according to Gastón et al., 2009) and is considered of great importance in the maintenance and recovery (e.g. after fire) of Mediterranean forests (Pérez-Bejarano et al. 2010). In nurseries, both species are grown in containers before being transplanted to the soil and the substrate in which the seedling has been growing until that moment is transferred to the natural soil. Environmentalists are reluctant to transfer alien materials to native soils in preserved environments because these materials cause transient alterations of soil characteristics (Doan et al. 2013). For this reason, whenever possible, nurseries should consider to propagate native species in the containerized native soil (e.g. *P. angustifolia* which is propagated by seed germination). Nevertheless, in some circumstances, such as in clonal propagation through cuttings (e.g. some shrubs such as *R. officinalis*), the success of rooting should be ensured by using rooting media with adequate physical and chemical characteristics (e.g. peat or coir, Fornes et al. 2013; Mendoza-Hernández et al. 2014).

In plant nurseries, which provide seedlings for horticulture (vegetables), gardening (ornamentals) and reforestation (forest shrubs and trees), the sphagnum peat has been the organic material preferably used as a growth medium for more than 50 years

(Schmilevski, 2009). This is due to the optimal characteristics (high porosity, good aeration, high water-holding capacity, low nutrient content and physical stability) (Maher et al., 2008) of peat for containerized plant production. Nevertheless, the exploitation of peatlands is considered environmentally unacceptable since peat is a non-renewable resource and these ecosystems are natural heritage and included in conservation policies (Maltby and Proctor, 1996; Alexander et al., 2008). Additionally, the drainage of peat bogs leads to increased emissions of greenhouse gases such as CO_2, CH_4 and N_2O (Martikainen, 1996; Clearly et al., 2005; Ojanen et al., 2013). Hence, there is a continuous search for alternative materials to peat for growth medium formulations (Abad et al., 2001; Pryce, 1991). Successful alternative materials to peat are coir and composts (Carrión et al., 2008; Belda et al., 2013; Mendoza-Hernandez et al., 2014).

Most recently, biochar has attracted attention as substrate or as substrate constituent. Biochar is the product of the controlled pyrolysis of organic matter and its main use is as soil improver in agriculture, horticulture and environmental care (Lehmann and Joseph 2009). Its promotion is based on its capacity to sequester carbon and its subsequent contribution to the reduction of greenhouse gas emissions (Kammann et al., 2012; Fornes et al., 2015; Xu et al., 2016). In general biochar has higher bulk density, lower total porosity, higher total air space and lower water-holding capacity than peat or coir, the magnitude of the difference depending on the biochar particle size (Fornes et al., 2017). This fact will reflect in the physical properties of growth media composed of mixes of biochar with peat (or any other material) at different proportions.

Biochar has been intensely assayed in the last few years for vegetable (Akhtar et al. 2014; Dunlop et al. 2015; Petruccelli et al. 1015; Fornes et al. 2017), ornamental (Zhang et al. 2014; Fornes and Belda 2018), forest (Belda et al. 2016; Cho et al., 2017; Di

Lonardo et al. 2017; Sarauer and Coleman, 2018) and soil-remediation-focused (Sáez et al. 2016) seedling production. Particularly, the effect of biochar addition in forest systems (both on soil and on plant growth) has been recently reviewed and meta-analysed by Thomas and Gale (2015). These authors conclude that 'trees in general show strong positive growth responses to biochar in a range of ecological systems and soil conditions'. Nevertheless, they advise against extrapolation of their results because the covered studies are mainly short-term pot experiments and it is likely that neutral or negative results are under-reported. Moreover, a great number of studies on the effects of biochar on forest species have been conducted in containerized systems using soilless growth media. For instance, Sarauer and Coleman (2018) found that biochar negatively affected the growth of Douglas-fir seedlings and they attributed this effect to the pH increase caused by biochar. Nevertheless, Dumroese et al. (2018) successfully replaced 25% to 50% of peat with different biochars to grow *Pinus ponderosa* seedlings. Cho et al. (2017) found that biochars from wood chips and rice husk could substitute 20% of the growth medium (peat:perlite:vermiculite; 1:1:1) of *Zelkova serrata* without causing negative effects but that other biochars from pine corn or crab shell strongly decreased seedling growth. This was indicative that the raw material used to manufacture the biochar affects its characteristics and the response of plants to it. Belda et al. (2016) found that the plant response to biochar also depends on the species. They found a positive effect of biochar (up to 50% in a mix with coir) on the growth of myrtle but no effect was found on the growth of mastic.

Peculiar to biochar are its alkalinity and its low nutrient content (Bargmann et al. 2013; Fornes et al. 2015). Among nutrients, N and P availability are particularly sensitive to the presence of biochar in the growing media. The high C:N ratio of many biochars suggests that N immobilization can occur in the growth medium in the presence of chars due to an stimulation of microbial activity, which would induce a N deficiency in the plant (Atkinson et al. 2010). Although it has also been argued that the recalcitrant nature of the carbon in biochar might limit the risk of N immobilization, (Chan and Xu 2009), this risk cannot be overlooked. In fact, Fornes et al. (2015) reported a strong N immobilization by biochar in an incubation experiment. Bargmann et al. (2014) compared physico-chemical adsorption of nitrate versus microbial consumption as possible mechanisms of N immobilization induced by hydrochar (a char obtained through hydrothermal carbonization) and concluded that the latter was the main responsible for this effect. The alkalinity and nutrient fixing capacity of some biochars might affect nutrient availability, mainly that of P, which precipitates as Ca phosphate at pH above 8.5, and that of Fe (Mukherjee and Zimmerman, 2013; Sarauer and Coleman, 2018). Attempts have been made in order to adjust biochar pH to neutral or slightly acidic values (Fornes and Belda 2017) and to enrich biochar with mineral elements with the aim to produce a new range of slow release fertilizers (Yao et al. 2015; Gwenzi et al. 2018). In any case, the effects of biochar on nutrient (e.g. N and P) availability when added to soils or to soilless growth media are complex as they involve not only abiotic (pH, EC, CEC, nutrient supply) but also biotic factors (alteration of the microbial population and changes in microbial activity) (Atkinson et al. 2010).

Considering that nurseries must produce healthy plants with well-developed shoot and root systems, the aim of this study was to prove whether biochar, either as in the raw-alkaline-nutrient-poor version or as in the acidified-phosphorous-and-nitrogen-rich variant and used as peat-based or native-soil-based growth medium constituent, improves rooting and growth of *Rosmarinus officinalis* L. cuttings, and growth of *Phillyrea angustifolia* L. seedlings.

Materials and methods

Plant material

Two species were used in the experiments: *Phillyrea angustifolia* L. and *Rosmarinus officinalis* L. Four-month-old seedlings of *Phillyrea* (about 5 cm in length with four to six leaves) were obtained from the nursery of L'Albufera Natural Park (Valencia, Spain). Cuttings of *Rosmarinus* (about 5 cm in length, obtained from lateral or terminal buds of mother plants) were supplied by TENISPLANT, S.L. (Picassent, Spain).

Description of biochars, peat and soil

Biochar (BCH; particle size <6 mm) was purchased from Piroeco Bioenergy S.L. (Malaga, Spain). It had been produced from holm oak by slow pyrolysis at 650ºC and atmospheric pressure. The residence time in the reactor chamber had been 12-18 h.

Peat was a limed and slightly fertilized sphagnum moss peat (Neuhaus N3®, Klasmann-Deilmann GmbH, Geeste, Germany).

The soil for this study was taken from La Devesa of the Albufera Natural Park (Valencia, Spain) and it is described as Calcaric Arenosol (Sanchís et al. 1986). It was sandy in texture with 96% sand, 2% silt and 2% clay. This is the soil that the park rangers ordinarily dig for the park nursery (39º 21' N, 0º 19' W).

The main physico-chemical properties and available (water soluble) nutrient content of BCH, peat and soil are presented in Table 1. Both soil and BCH were strongly alkaline materials whilst peat was acidic. EC was low in the three materials, the lowest being the soil. Both peat and BCH were rich in OM (77% to 96%), whilst soil had very low OM content (1%). With respect to the available nutrient content, it was low in the three materials. In this respect, with the exception of K content, which was larger in soil than in peat, soil was the poorest. BCH and peat had similar N and P contents but BCH was richer in K than peat and peat was richer in Ca, Mg, S, and Fe than BCH. A-BCH-I and A-BCH-II had more N and P than BCH as these nutrients were supplied with the acidifying solutions (more in A-BCH-II than in A-BCH-I). Additionally, acidified biochars had more soluble Ca, Mg, and Fe than BCH (more in A-BCH-II than in A-BCH-I). This was likely due to the decrease of pH caused by acidification, as Fe and Mg solubility increases at pH values below 8.0 (in the alkaline range) (Peterson et al. 1981) and Ca could had been released from the $CaCO_3$ usually present in biochars (Fornes et al. 2015).

Physical, physico-chemical and chemical characterization of biochars, peat, soil and growth media

Biochars, peat and soil were chemically characterized whilst growth media were characterized both physically and chemically. The characterisation was carried out following the European Standards (EN) for soil improvers and growth media as described by Belda et al. (2016).

Particle size was determined on 200-mL air-dried aliquots. Particles of different sizes were separated using an electromagnetic vibratory shaker, for 10 min, with sieves of square mesh sizes of 0.125, 0.25, 0.50, 1, 2, 4, 8 and 16 mm. The material collected in each sieve was weighed and expressed as a percentage by weight of the whole sample. Coarseness index (CI) was calculated as the accumulated percentage in weight of particles larger than 1 mm. Bulk density, water capacity and total water-holding capacity were determined using loosely-packed cores and following the methods described in EN 13041 (1999), using steel cylinders of 40 mm height and 82.3 mm internal diameter (210 mL). Shrinkage was calculated as the percentage loss of bulk volume after drying the material contained in the cylinder at 105ºC. Total pore space is the percentage of the material volume that can be filled with water. Air capacity is the difference −in percentage by volume− between total pore space and the moisture content at a suction of 1 kPa (EN 13041, 1999).

Available (water soluble) nutrient content, pH and electrical conductivity (EC) were determined in a 1:5 (v:v) material:water suspension, following the European Standards (EN 13652, 2001, EN 13037, 1999, and EN 13038, 1999, respectively) for soil improvers and growing media. Nitrogen was analyzed using a TOC-V CSN analyser (Shimadzu) and P, K, Ca, Mg, S, and Fe were analysed by inductively coupled plasma-optical emission spectroscopy (ICP-OES; ICAP 6500 DUO+ONE FAST, Thermo Scientific). Mineral concentrations were expressed on a volume basis as suggested by Blok et al. (2008) for growth media.

For organic matter (OM) and ash (MM) the material was dried at 105ºC and ashed at 450ºC. OM was calculated as the mass loss in percentage.

For each material or growth media, one aliquot was taken from three separate batches (replicates) and determined separately in order to account for lack of uniformity. Besides each determination was replicated three times.

Biochar acidification. Titration curve and selection of the optimal acidifying solution

Water content of the biochar at saturation (SV; saturation volume) was previously determined as follows: three aliquots of 200 mL of biochar were saturated with water for 15 hours. The biochar was then drained for 10 hours. The gravimetric difference between dry and water-saturated-drained biochar showed that biochar could hold a SV=467 mL of water per L of material at saturation. With this datum and based on previous experiences of acidification of biochar with nitric acid (Fornes and Belda 2017), a titration curve was prepared by mixing 1 L of dry biochar with 467 mL of several acidifying solutions. The acidifying solutions contained nitric acid and phosphoric acid with the aim of adding N and P to the biochar, thus increasing its fertilizing capacity on top of decreasing the pH. The composition (concentration of HNO_3 and H_3PO_4), pH and H^+ and supply of N and P of each solution are included in Table 2. The acidified biochars were dried at room temperature for 15 days. After acidification biochar pH and electrical conductivity (EC) (soilless growth media are required to have low EC in order to avoid salinity and other stresses to plants) were determined as indicated above. Based on the most adequate pH and EC values, we selected the solution with 0.2M HNO_3 + 0.1M H_3PO_4 (resulting in a biochar [A-BCH-I] with pH=7.0 and EC=1.21 dS m^{-1}; Table1) for the leaching study and for experiment I, in which high proportions of biochars were assayed. For experiment II, in which low proportions of biochar were assayed, we selected the solution with 0.6M HNO_3 + 0.3M H_3PO_4 (resulting in a biochar [A-BCH-II] with pH=5.3 and EC=3.85 dS m^{-1}; Table1).

Leaching experiment of the acidified biochar

To study how NO_3^- and the PO_4^{3-} were retained by the biochar, a column experiment was carried out as described by Fornes et al. (2010). Briefly, three methacrylate columns (40 cm height and 5.3 cm internal diameter), fastened in vertical position, were filled with 883 mL of the biochar acidified with the 0.2M HNO_3 + 0.1M H_3PO_4 solution.

Fractionated volumes (0.25 x SV) of distilled water were sequentially poured through the columns and the leachates separately collected and analyzed for NO_3^- and the PO_4^{3-}. Analyses were carried out simultaneously to the collection of fractions using reflectoquant technology (Merck) with a reflectometer RQflex 10 Reflectoquant, the corresponding bar-code strips for calibration and the test strips for nutrient quantification, following the manufacturer's instructions. The total volume of water poured through the columns was 1648 mL (16 x 103 mL).

Plant growth experiments: treatments and experimental design

Two experiments were conducted to produce *Rosmarinus* and *Phillyrea* seedlings under the same conditions as nurseries.

Experiment I: rooting and growth of Rosmarinus cuttings.

Treatments consisted of mixing BCH or A-BCH-I with peat. The assayed mixes were BCH:Peat and A-BCH-I:Peat in the proportions (v:v) of 80:20, 40:60 and 0:100 (control). The experiment was conducted in a glasshouse at a commercial nursery (TENISPLANT, S.L.) located in Picassent, Spain (39º 33' N, 0º 44' W) from March to June 2017. Three 30-cell plastic rooting trays (cell volume = 62 mL) were filled with each of the mixes and distributed in a random block design (three replicates per treatment; a total of 3 rep. x 6 treat. = 18 trays). One cutting per cell was placed in the substrate. Cuttings were irrigated using a microsprinkler system (performance of 36 L h^{-1} m^{-2}) at a regime of 5 min once a day, resulting in 0.6 L $tray^{-1}$ day^{-1}. Rooting percentage and shoot dry weight and root visual score (root size was rated visually on a 1–4 scale, value 1 representing roots which do not reach the surface of the substrate and value 4 representing a root system forming compact mesh and colonizing the whole substrate, according to Fornes et al. 2007) were recorded three months after planting.

Experiment II: growth of Phillyrea seedlings.

Treatments consisted of mixing BCH or A-BCH-II with soil. Assayed mixes were BCH:Soil and A-BCH-II:Soil in the proportions (v:v) of 30:70, 20:80, 10:90 and 0:100 (control). Each of these growth media was fertilized with 5 g L^{-1} of a controlled release fertilizer (Osmocote®Plus, 6 months). The experiment was carried out from February to

July 2017 in a net protected tunnel located in the nursery of L'Albufera Natural Park (39º 21' N, 0º 19' W). Thirty pots of 300 mL capacity were filled with each growth medium and one seedling was transplanted to each pot. Pots were distributed in a random block design of 3 blocks (replicates) with 10 pots each. Plants were irrigated with microsprinklers when needed. The experiment was over when the plant size was appropriate for transplant to the soil, six months after being transferred to the pots. Relevant plant growth parameters (n◦ of leaves, shoot length and dry weight, and root length and dry weight) were recorded.

In both experiments, oven-dried leaf tissue of *Rosmarinus* and *Phillyrea* was finely ground for analysis. Nitrogen was determined by burning the material at 1020ºC in an elemental analyser (EuroVector EuroEA 3000). The concentration of other elements (P, K, Ca, Mg, Fe) was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; ICAP 6500 DUO+ONE FAST, Thermo Scientific) after microwave (ETHOS1, Milestone) assisted HNO_3/H_2O_2 digestion. Vector analysis diagrams of foliar nutrients (Haase and Rose, 1995; Headlee et al. 2014) were obtained for each biochar and dose in each plant species. In this case, shoot biomass, leaf nutrient concentration and nutrient content data were normalized relative to the 0% biochar treatments.

Data analysis

Factorial ANOVAs were performed to determine significant effects of the biochar type and the dose on growth medium characteristics and plant growth. When significant differences were found, the Tukey test at $P \leq 0.05$ was carried out to establish significant differences between means. Statistical analyses were performed using the Statgraphic Plus for Windows 5.1 statistical package (Statistical Graphics Corp., 2000).

Results

Leaching of NO_3^- and PO_4^{3-} of the acidified biochar in the column experiment

Fig. 1 shows the accumulated leached NO_3^- and PO_4^{3-} (% of the initial amount) from the biochar which was acidified with 0.2M HNO_3 + 0.1M H_3PO_4, with the consecutive fractions of water poured into the column. NO_3^- was easily washed, which indicated a loose adsorption to the solid matrix. On the contrary, PO_4^- was strongly adsorbed by the biochar (only 6% of the initial content was washed after pouring 4 x SV).

Effect of biochars on the properties of peat or soil based growth media

Table 3 and 4 show the physical and physico-chemical (pH and EC) characteristics of the growth media assayed in experiments I and II.

As acidification did not affect the physical properties of biochar, BCH and A-BCHs affected in the same way the physical parameters of the growth media in both experiments.

In experiment I (Table 3), the values of the physical parameters in the mixes were intermediate between those of peat and those of BCHs, them being closer to one or to the other depending on the proportion of each component in the mix. In this way, as BCHs increased in the mix, Db, D_P and Vair increased and P_T, WHC, Vwater and shrinkage decreased in comparison to the peat control. With respect to pH, it increased in both BCH and A-BCH-I containing media this increase being more prominent for BCH than for A-BCH-I. On the other hand, BCH decreased EC whilst A-BCH-I increased EC in the growth media when compared to peat.

Also in experiment II (Table 4) the values of the physical parameters in the mixes were intermediate between those of soil and those of BCHs. In this case, as the properties of the soil were very different to those of the peat, the changes caused by BCHs in the mixes with soil were different to those caused in the mixes with peat. For instance, as BCHs increased in the mix with soil, Db and D_P decreased and P_T, WHC, Vair and Vwater increased. In this case, shrinkage was not affected by BCHs. With respect to pH, as soil was strongly alkaline (Table 1), both BCH and A-BCH-II decreased it in the media this decrease being more prominent for A-BCH-II than for BCH. Besides, both BCH and A-BCH-II increased EC in the media, more so the latter than the former.

Table 5 and 6 show available (water soluble) nutrient content of the growth media of experiments I and II. In both types of growth media (organic in experiment I and mineral in experiment II) the contribution of significant amounts of N and P by the acidification treatments made those mixes containing A-BCHs richer in these nutrients than the mixes containing BCH, although for P the difference was smaller than for N due to its low solubility. In the peat-based media the amount of N and P increased as the proportion of chars increased in the mix, this effect being small in the case of BCH and remarkable in the case of A-BCH-I (Table 5). In the soil-based media the amount of these nutrients increased as A-BCH-II increased in the mix but there was no dose effect in the case of BCH (Table 6). Potassium increased in both types of growth media as the proportion of char increased, regardless of whether it had been acidified or not, whilst sulphur increased only in the soil-based media (experiment II; Table 6). Iron increased with the addition of A-BCHs in both types of growth media but not with the addition of BCH. Calcium and magnesium contents were similar in all growth media.

Rooting of cuttings, plant growth, plant nutrient status and foliar nutrient vector analyses

Table 7 shows the response of *Rosmarinus* cuttings to organic growth media containing mixes of BCH or A-BCH-I with peat (experiment I). The most outstanding effects were that BCH improved the rooting of cuttings at all assayed doses whilst A-BCH-I improved rooting only at the medium dose of 40% but had no effect at the high dose of 80%. Apart from the effect on rooting, rooted cuttings grew better in the A-BCH-I containing media, even at the high dose of 80%, than in the BCH containing media, and in all cases they grew better than in the peat control medium. With respect to the nutritional status of the plants, the presence of char in the media reduced the concentration of N in plants, although there was not a dose effect. Besides, plants grown in A-BCH-I containing media had higher concentration of N and P than those grown in BCH containing media. Chars did not affect the concentration of the other elements. Vector analyses of foliar nutrients of all the treatments with biochars (Fig. 2 a, b, c, d) indicate that the total content of all the nutrients increased in leaves with seedling growth with respect to the peat control. Compared to the control, the 40% BCH treatment (Fig. 2 a) showed a dilution effect for N, P and K whilst Mg and mainly Ca concentrated in the tissues. For the 80% BCH treatment (Fig. 2b), N, P and Ca were slightly diluted in the leaves whereas Mg was concentrated. For the A-BCH-I treatments, an increase in the concentration of P and K in the leaves was observed, but only in the dose of 40% (Fig. 2 c). The concentration of other nutrients in plants growing in this medium and the concentration of all the nutrients in plants growing in the 80% A-BCH medium were similar to that in plants growing in the peat control.

Table 8 shows the response of *Phillyrea* seedlings to mineral-based growth media containing mixes of BCH or A-BCH-II with soil (experiment II). BCH increased shoot growth, both in length and mass, no matter the dose. The effect on root growth was to increase its mass but shorten its length. A-BCH-II had no significant effect on plant growth. Char type or dose did not significantly affect plant nutritional status. Exceptionally, a significant increase in Ca was recorded for media containing either BCH or A-BCH-II. The nutrient vector analyses for the three doses of BCH (Fig. 3 a, b, c) showed a slight dilution effect for P (only doses of 20 and 30%) whilst Mg and mainly Ca concentrated in the tissues. With respect to the A-BCH-II treatments (Fig. 3 d, e, f), the clearest effects were the concentration of Ca in tissues of plants grown at the 10% and 30% doses, but not at the 20% dose, and the concentration of K and P in tissues of plants grown at the 10% dose and that of Mg at the 30% dose.

Discussion

This study indicates that biochar is adequate as growth media constituent for rooting and growth of *Rosmarinus* cuttings, even when used in large proportions, and also for the growth of *Phillyrea* seedlings. To our knowledge, there is no published information about the effects of biochar on rooting of *Rosmarinus* cuttings though it has been successfully tested in other species such as poplar (Headlee et al. 2014). The situation for *Phillyrea* is similar, since, in this respect, we can only refer to the study of Di Lonardo et al. (2017). Besides, biochar acidified with HNO_3 and H_3PO_4 behaved differently in the two experiments conducted. In experiment I *Rosmarinus* grew best in substrates containing acidified biochar whilst in experiment II *Phillyrea* grew best in substrates containing alkaline biochar. The reasons for this should be sought in the different conditions of each experiment: 1) different base material in the substrate (acid peat in experiment I and alkaline soil in experiment II); 2) biochar differently acidified (A-BCH-I in experiment I and A-BCH-II in experiment II); 3) different fertilization (no basal fertilization in experiment I and basal fertilization with controlled release fertilizer in experiment II); 4) different species used (cuttings of *Rosmarinus* that must take root before growing in experiment I and *Phillyrea* seedlings in experiment II) each with its own requirements.

The main factors of growth media that affect plant response are the physical properties (i.e. porosity and air-water relationships), the physico-chemical characteristics (i.e. pH, EC, CEC) and the nutritional status (i.e. nutrient content and availability to plants). Among them, the physical properties are considered more important than the chemical ones because the latter can be modified during plant growth yet the former are not readily managed during the growth cycle, particularly when plants are grown in a limited space (e.g. pots). In fact, adequate ranges (AR) have been proposed for the most relevant physical and physico-chemical properties for growth media devoted to rooting of cuttings in pots (Db = 300-800 Kg m^{-3}; P_T > 85%; Vair = 15-40%; Vwater = 20-60%; pH = 4.5-6.5; EC = 0.05-0.20 dS m^{-1}; Maronek et al., 1985). Nevertheless, the relevance of the physical properties of rooting media is debatable since some studies showed significant effects of these factors on rooting performance (Harfouche et al. 2007, on poplar) and others did not find significant effects (Tate and Page 2018, on *Santalum austrocaledonicum*). In the latter case, the authors assayed a variety of rooting media containing scoria and mixes of perlite, vermiculite and peat, all of them having adequate physical properties.

In general, peat-based substrates (experiment I; Table 3) meet or nearly meet the most important (P_T, Vair and Vwater) physical requirements for specific media for rooting of cuttings. Even so, biochars improved the air/water ratio of the peat substrate, which could explain the improvement of rooting and root growth caused by the biochars (Table 7). Mendez et al. (2015) found that biochar, when mixed with peat, increases air space, water-holding capacity and total porosity. In our study, biochar also increased air volume but decreased water-holding capacity and total porosity. This discrepancy might be due to the fact that these authors used peat and biochar of particle size different from that of our study and it is well known that particle size affects the physical properties of the materials (Abad et al. 2005). Another reason which explains the better behavior of the cuttings in the biochar containing substrates than in the non-containing ones might be the change of pH in the growth media. pH is relevant because species might be calcifuge or calcicole (Lee 1998). Particularly, *Rosmarinus officinalis* has been defined as a non-strict calcicole (Gastón et al. 2009), which means that it prefers calcareous soils but is also able to grow in slightly acidic ones. Hence, the pH increase from the acid value of peat (5.6) to the neutral (7.0 to 7.2) or alkaline (7.6 to 8.7) of the A-BCH-I and BCH containing media, respectively, could have contributed to the indicated improvement. The failure of the 80% A-BCH-I treatment in improving the rooting of the cuttings might be related to an excessive increase of EC in the medium, which counteracted the benefits of improved aeration and pH caused by biochar. In fact, a negative correlation between EC and percentage of rooted cuttings of *Rosmarinus* (Mendoza-Hernández et al. 2014), *Euonymus*, and *Lavandula* (Fornes et al. 2013) has been demonstrated.

With respect to the different effect of BCH and A-BCH-I on shoot growth, it must rely on nutritional factors since both biochars affected the physical properties of the media similarly (Table 3). As the substrate did not receive basal fertilization, the only nutrients sustaining plant growth were those supplied by the biochars and the peat (Table 1). The most obvious difference between BCH and A-BCH-I was the high N and P amount (Table 5) and the low pH (Table 3) that A-BCH-I supplied and conferred, respectively, to the media. Additionally, we must consider the solubility of these nutrients and the possibility that they might be leached with irrigation. In this regard, pH is relevant because nutrients are more or less soluble (available) depending on pH (Peterson 1981). Nevertheless, in organic media nitrate solubility is high at any pH value (Peterson 1981). Also Gai et al. (2014) indicated that nitrate is highly soluble and does not bind efficiently to activated carbon or biochar in aqueous solutions. This was confirmed for biochar in our column experiment, in which NO_3^- was easily leached (Fig. 1). For peat it has also been indicated that it does not retain nitrate efficiently (Bigelow et al. 2001). Hence, in these media the N-NO_3^- is prone to leaching through irrigation. Nevertheless, although leachates were not measured in our experiment, it would appear that they were low due to the short wetting regime applied (see 'Plant growth experiments: treatments and experimental design' in Materials and methods section). Moreover, contrary to our findings, Altland and Locke (2012) found that biochar added at low doses to a substrate of peat mixed with perlite reduced the nitrate content in leachates. Contrariwise phosphate was strongly retained by biochar in our column experiment (Fig. 1), inducing us to think that its availability for plants in the short term must be low. In fact, Peterson (1981) indicated that the solubility of P decreases markedly at pH higher than 5.5-6.5 in organic media. In accordance with our results, Sarauer and Coleman (2018) also found that phosphorous availability was low on peat based growing media amended with biochar. They justified this effect because phosphorous likely precipitated with Ca due to the pH increase caused by biochar. Laird et al. (2010) studied the leaching of nutrients from an agricultural soil amended with swine manure as a source of nutrients and biochar in a column experiment. They found that PO_4^{3-} leaching was reduced by more than 80% due to biochar whilst, although total N (mainly N-NH_4^+ and organic-N) leaching was reduced by biochar, NO_3^- leaching was not reduced by the biochar amendment. These authors indicated that the surface anionic charge sites (mostly carboxylic and phenolic groups [Liang et al. 2006]) typical of aged biochars prevented NO_3^- from being retained by these materials. Diversely, Gwenzi et al. (2018) manufactured a granulated biochar-based slow release fertilizer impregnated with nutrients and covered with a starch-PVA binder, which retained significant amounts of NO_3^- and PO_4^{3-}. However, in this instance, it remained unclear whether the increased retention was due to the biochar or to the binder. Joseph et al. (2018) determined that co-composting biochar with other nutrient rich organic materials provided a composted biochar that held and retained nutrients better than the non-composted biochar. They argued that the composting process chemically modified the surface of the biochar by covering it with carbon reactive groups, which made it more prone to retain nutrients than the non-composted.

In our experiment, despite the supply of N and P by A-BCH-I, N, P and K contents in shoots (Table 7) were below those considered as sufficient range (SR) for *Rosmarinus* (2.09-2.52% for N; 0.26-0.35% for P; 2.36-2.55 for K) whilst Ca was above SR (0.48-0.69%) and Mg within the range (0.17-0.40%) (Mills and Jones 1996). A similar finding was reported by Mendoza-Hernández et al. (2014) assaying mixes of compost and vermicompost with peat as substrates for rooting and growing cuttings of *Rosmarinus officinalis*. This does not necessarily mean that there was a limiting nutrient supply to the plants since the above indicated SR correspond to adult plants and not to small fast growing seedlings as the ones used in our experiment. The feeding potential of growth media might be deduced from the vector analysis of the seedling nutrient content following the interpretation suggested by Haase and Rose (1995). The increase in total nutrient content and in the concentration of Ca (Fig. 2a) and Mg (Fig. 2b) indicates that peat was deficient in these nutrients and that BCH supplied them and increased their availability probably due to the increase of pH (Peterson et al 1981). The decrease in N, P and K concentration joint to the increase in total content of these three nutrients indicates that they were not limiting in the media and that a dilution effect or a translocation to the roots occurred as the seedling grew. Considering the effect of A-BCH-I, the supply of N and P, together with the change in pH, led to a large increase in seedling growth. This effect might be supported by the large increase in P availability, and, to a lesser extend, in K availability, as indicated by their increase in the plant total content and in tissue concentration (Fig. 2 c). As phosphate was initially strongly retained by biochar, the increase in its availability could had been due to a direct action of roots through the exudation of organic compounds (organic acids, chelates) that acidified the medium and dissolved the immobilized phosphates or to an indirect action of roots through the stimulation of phosphate dissolving microbial populations. Nevertheless, these possibilities, which were profusely studied by other authors (see Otani and Ae 2001, and Randall et al. 2001), have not been explored in the present study.

In experiment II, *Phillyrea angustifolia* was grown in alkaline sandy soil based growth media and it was found that alkaline biochar BCH stimulated plant growth whereas acidified biochar A-BCH-II did not. The effect of biochar on the growth of *Phillyrea* has been scarcely studied. Di Lonardo et al. (2017) did not find any effect on plant growth by the presence of an alkaline biochar in the growth media. They used a biochar similar to the BCH used in our study and they also grew the plants for a period of six months. Nevertheless, they used plants ten times taller than the ones used by us and, perhaps, the larger nutrient (both carbohydrates and minerals) reserves in the larger plants made them less susceptible to the growth media in the short term. Supporting this speculation, Thomas and Gale (2015) indicated that tree responses to biochar were especially pronounced at early growth stages. In general, many studies on the effects of chars on plant growth refer to their use as soil amendments (Gaskin et al. 2010; Spokas et al. 2012). Although results are contradictory, they raised a general agreement on the beneficial effects caused by biochar when applied to poor and to sandy soils (Haefele et al. 2011; Peng et al. 2011; Omil et al. 2013). Besides, in a previous study, Belda et al. (2016) grew myrtle and mastic, also Mediterranean shrubs typical of L'Albufera Natural Park, in mixes of the same soil with a biochar similar to the BCH assayed in the present study. They found that mixes with biochar improved the survival and growth of myrtle but were ineffective on mastic, indicating a species-dependent response. In the same way that for rooting media, some AR have been suggested for containerized plant cultivation for the main physical and physico-chemical properties (Db < 400 Kg m^{-3}; P_T > 85%; Vair = 20-30%; Vwater = 52-68%; pH = 5-6.3; EC = 0.75-3.49 dS m^{-1}; Bunt, 1988; Carmona and Abad, 2008). However, in this case, the comparison might be futile as the recommended values fit better for light organic or mineral media (e.g. peat, coir, perlite) than for heavy sandy soil media. In any case, the addition of biochars to the sandy substrate improved porosity, aeration, and water retention, independently of the biochar type (Table 4). Nevertheless, other factors must be the responsible for the differential effects of BCH and A-BCH-II because both biochars affected the physical properties of the media similarly. Nutrient availability did not seem to be the reason why A-BCH-II did not stimulate plant growth, since in experiment II growth media were fertilized with a controlled release fertilizer. In fact, media containing A-BCH-II were richer in available K, Fe and above all N and P. Actually, the pH in the A-BCH-II containing media was ideal to ensure the availability in the mineral soil of all nutrients, including P (Peterson 1981). Plants responded by concentrating the nutrients (mainly Ca) in the shoot tissues (Fig 3 d, f), indicating a close to luxury consumption without symptoms of toxicity in the indicated conditions in which seedling growth was not significantly increased (Haase and Rose 1995). Alternatively, BCH did not supply additional nutrients to the growth media (Table 6) but stimulated plant growth and leaf concentration of Ca and, to a lesser extend, Mg significantly (Table 8; Fig 3 a, b, c). pH is another factor to take into account. Although *Phillyrea* has been described as ‘indifferent’ in relation to the calcicole-calcifuge behavior (Gastón et al. 2009), the plant material used in our experiment was undoubtedly well adapted to alkalinity since the native soil of the L’Albufera natural park, where *Phillyrea* grows native, has a pH value of 9.6 (Table 1). Moreover, some studies indicated alkaline-loving traits in *Phillyrea*. For instance, De Lucia et al. (2013) reported that, in compost amended soil, *Phillyrea* grew the better the more alkaline the soil was. From this point of view, it was significant that A-BCH-II conferred to the substrates pH values (6.6-7.5) close to neutrality, far from that of the strongly alkaline native soil, whereas BCH maintained the alkalinity in the growth media (pH = 9.1-9.5) (Table 4). Other possible factors that might have affected growth in *Phillyrea* in our experiments (e.g. potential phytotoxic compounds in A-BCH-II; changes in soil microbial populations, etc.) have not been evaluated in the present study.

Conclusion

Although our experiments correspond to a short term study (three months for the *Rosmarinus* experiment and six months for the *Phillyrea* experiment), from our results it might be concluded that biochar contributes to improve root formation and growth of both *Rosmarinus* and *Phillyrea* regardless of whether the growth medium is mainly organic or mineral. In addition to this, shoot growth of both species was stimulated by biochar. The use of biochar produces, hence, stronger plants, which might better resist the stress caused by the transplant to the final soil (garden, forestry, etc.). Regarding biochar acidification with mineral acids as a means to adjust pH and supply growth media with nitrogen and phosphate, this treatment might not consistently improve biochar. Nitrate might be easily leached from the growth media whilst phosphate might be strongly retained, which would question their actual availablility to plants.

For all these reasons, we recommend including biochar, without any additional treatment, in the formulation of rooting and growth media in nurseries devoted to the propagation of forestry species for landscaping, gardening or reforestation purposes. In this sense, an additional advantage of biochar is that it cannot be considered an alien material when added to forest soils in the final transplant to the ecosystem for it has been incorporated into these soils for millennia due to natural fires or anthropogenic activity (Forbes et al. 2006). However, as biochars vary in their characteristics due to different factors (feedstock, processing conditions, etc.) and each species has its particular requirements for growth, it is advisable to test each biochar/plant pair before undertaking a propagation program on a large scale.

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Table 1 Main physico-chemical properties and available (water soluble) nutrient content of the raw biochar (BCH), the acidified biochars (A-BCH), the peat, and the soil used for the preparation of growth media

Parameter	BCH	A-BCH-I (experiment I)	A-BCH-II (experiment II)	Peat	Soil
pH	9.3	7.0	5.3	5.6	9.6
EC (dS m^{-1})	0.50	1.21	3.85	0.75	0.10
OM (%)	79	78	77	96	1
N (mg Kg^{-1})	50	4100	12150	60	10
P (mg Kg^{-1})	20	270	810	13	7
K (mg Kg^{-1})	1070	1055	1060	49	150
Ca (mg Kg^{-1})	40	70	120	244	12
Mg (mg Kg^{-1})	10	20	60	62	3
S (mg Kg^{-1})	80	85	86	267	<0.01
Fe (mg Kg^{-1})	2	10	30	10	<0.01

EC: electrical conductivity; OM: organic matter

Table 2 Protons, pH, electrical conductivity (EC), nitrogen and phosphorous supplied to biochar by acidifying solutions of different concentrations of HNO_3 plus H_3PO_4. The volume of the applied acidifying solution was equivalent to the water retention of biochar at saturation (SV=467 mL L-biochar^{-1})

HNO_3:H_3PO_4	H^+ (meq L-biochar^{-1})	pH	EC (dSm^{-1})	N-NO_3^- (mg L-biochar^{-1})	P-PO_4^{3-} (mg L-biochar^{-1})
0:0	0	9.3	0.50	0	0
0.1M:0.05M	122	7.5	0.65	654	724
0.2M:0.1M	244	7.0	1.21	1308	1448
0.3M:0.15M	366	6.7	1.90	1962	2172
0.4M:0.2M	488	6.3	2.41	2616	2896
0.5M:0.25M	610	6.0	3.20	3270	3620
0.6M:0.3M	732	5.3	3.85	3924	4344

Table 3 Physical and physico-chemical properties of the growth media produced by mixing raw biochar (BCH) or acidified biochar (A-BCH-I) with peat (experiment I)

Material	Dilution (% v:v)	Db (Kg m^{-3})	Dp (Kg m^{-3})	P_T (% v:v)	WHC (g L $media^{-1}$)	Vair (% v:v)	Vwater (% v:v)	Shrinkage (%)	pH	EC (dS m^{-1})
BCH:Peat	80:20	310	1610	83	200	29	54	12	8.7	0.49f
	40:60	195	1578	87	375	24	63	17	7.6	0.66d
	0:100	72	1543	91	580	21	71	22	5.5	0.78c
A-BCH-I:Peat	80:20	308	1607	82	210	27	55	11	7.2	1.43a
	40:60	190	1580	86	370	22	64	16	7.0	1.24b
	0:100	70	1540	90	583	20	70	22	5.6	0.75c
Main effects										
Material										
BCH		192	1577	87	385	25	63	17	7.3a	0.64
A-BCH-I		189	1576	86	388	23	63	16	6.6b	1.14
Dilution										
80:20		309a	1609a	83c	205c	28a	55c	12c	8.0a	0,96
40:60		193b	1579b	87b	373b	23b	64b	17b	7.4b	0.95
0:100		71c	1542c	91a	582a	21b	71a	22a	5.6c	0.77
Statistical significance										
Material		ns	ns	ns	ns	ns	ns	ns	***	***
Dilution		***	***	***	***	*	***	***	***	**
M x D		ns	ns	ns	ns	ns	ns	ns	ns	***

Db: bulk density; Dp: particle density; P_T: total pore space; WHC: water holding capacity; Vair: air volume; Vwater: water volume; EC: electrical conductivity.

ns, *, **, *** indicate not significant, statistically significant differences at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Values in the same column with different letters are statistically different at $P \leq 0.05$ (Tukey test)

Table 4 Physical and physico-chemical properties of the growth media produced by mixing raw biochar (BCH) or acidified biochar (A-BCH-II) with soil (experiment II)

Material	Dilution (% v:v)	Db (Kg m^{-3})	Dp (Kg m^{-3})	P_T (% v:v)	WHC (g L $media^{-1}$)	Vair (% v:v)	Vwater (% v:v)	Shrinkage (%)	pH	EC (dS m^{-1})
BCH:Soil	30:70	1250	2500	54	462	9	45	0	9.1	0.22
	20:80	1385	2561	48	429	7	41	0	9.4	0.16
	10:90	1560	2620	42	429	4	38	0	9.5	0.12
	0:100	1605	2645	41	430	3	38	0	9.6	0.10
A-BCH-II:Soil	30:70	1300	2495	55	475	9	46	0	6.6	1.49
	20:80	1410	2565	47	423	6	41	0	6.9	1.03
	10:90	1580	2610	41	431	4	37	0	7.5	0.58
	0:100	1600	2650	40	432	3	37	0	9.5	0.10
Main effects										
Material										
BCH		1450	2582	46	438	6	41	0	9.4a	0.15a
A-BCH-II		1473	2580	46	440	6	40	0	7.6b	0.80b
Dilution										
30:70		1275c	2498c	55a	469a	9a	46a	0	7.9d	0.86a
20:80		1398b	2563bc	48b	426b	7ab	41b	0	8.2cd	0.60b
10:90		1570a	2615ab	42c	430b	4b	38b	0	8.5bc	0.35c
0:100		1603a	2648a	41c	431b	3b	38b	0	9.6a	0.10d
Statistical significance										
Material		ns	ns	ns	ns	ns	ns	ns	**	***
Dilution		***	**	**	*	*	*	ns	**	***
M x D		ns	ns	ns	ns	ns	ns	ns	ns	ns

Db: bulk density; Dp: particle density; P_T: total pore space; WHC: water holding capacity; Vair: air volume; Vwater: water volume; EC: electrical conductivity.

ns, *, **, *** indicate not significant, statistically significant differences at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Values in the same column with different letters are statistically different at $P \leq 0.05$ (Tukey test)

Table 5 Available (water soluble) nutrient content (mg L growth media^{-1}) of the growth media produced by mixing raw biochar (BCH) or acidified biochar (A-BCH-I) with peat (experiment I)

Material	Dilution (% v:v)	N	P	K	Ca	Mg	S	Fe
BCH:Peat	80:20	14	5	277	14	3	24	0.7c
	40:60	7	3	140	15	4	22	0.3c
	0:100	4	1	3	17	4	19	0.7c
A-BCH-I:Peat	80:20	1060	75	273	22	6	26	2.7a
	40:60	532	37	138	19	5	22	1.7b
	0:100	5	1	2	18	4	18	0.7c
Main effects								
Material								
BCH		8b	3b	140	15	4	22	0.6
A-BCH-I		532a	38a	138	20	5	22	1.7
Dilution								
80:20		537a	40a	275a	18	5	25	1.7
40:60		270b	20b	139b	17	5	22	1.0
0:100		5c	1c	3c	18	4	19	0.7
Statistical significance								
Material		***	***	ns	ns	ns	ns	*
Dilution		***	***	***	ns	ns	ns	*
M x D		ns	ns	ns	ns	ns	ns	*

ns, *, *** indicate not significant, statistically significant differences at $P \leq 0.05$, $P \leq 0.001$, respectively. Values in the same column with different letters are statistically different at $P \leq 0.05$ (Tukey test)

Table 6 Available (water soluble) nutrient content (mg L growth media^{-1}) of the growth media produced by mixing raw biochar (BCH) or acidified biochar (A-BCH-II) with soil (experiment II)

Material	Dilution (% v:v)	N	P	K	Ca	Mg	S	Fe
BCH:Soil	30:70	16d	10d	270	17	4	8	0.2c
	20:80	17d	10d	261	18	4	5	0.1c
	10:90	16d	11d	250	19	5	3	0.1c
	0:100	16d	11d	240	19	5	0.01	0.01c
A-BCH-II:Soil	30:70	1180a	100a	264	25	9	8	3a
	20:80	790b	70b	257	23	8	6	2ab
	10:90	385c	40c	247	23	6	3	1b
	0:100	16d	11d	245	18	5	0.01	0.01c
Main effects								
Material								
BCH		16	11	255	18	5	4	0.1
A-BCH-II		593	55	253	23	7	4	1.5
Dilution								
30:70		598	55	267a	21	7	8a	1.6
20:80		404	40	259ab	21	6	6ab	1.1
10:90		201	26	249bc	21	6	3b	0.6
0:100		16	11	243c	19	5	0.01c	0.01
Statistical significance								
Material		***	***	ns	ns	ns	ns	*
Dilution		***	***	*	ns	ns	*	*
M x D		*	*	ns	ns	ns	ns	*

ns, *, *** indicate not significant, statistically significant differences at $P \leq 0.05$, $P \leq 0.001$, respectively. Values in the same column with different letters are statistically different at $P \leq 0.05$ (Tukey test)

Table 7 Rooting, growth, and leaf nutrient content (% dry weight) of rosemary cuttings grown in growth media produced by mixing raw biochar (BCH) or acidified biochar (A-BCH-I) with peat (experiment I)

Material	Dilution (% v:v)	Rooted cuttings (%)	Shoot dry mass (mg)	Root ball (VR score)	N	P	K	Ca	Mg	Fe
BCH:Peat	80:20	100a	75bc	3.0	1.52	0.14	1.61	1.30	0.44	0.009
	40:60	97a	89b	3.5	1.23	0.12	1.24	2.02	0.39	0.016
	0:100	77b	48cd	2.1	1.70	0.16	1.42	1.53	0.33	0.014
A-BCH-I:Peat	80:20	77b	140a	3.0	1.58	0.19	1.32	1.91	0.37	0.011
	40:60	90a	160a	4.0	1.54	0.23	1.96	1.36	0.36	0.018
	0:100	75b	43d	1.9	1.72	0.15	1.40	1.50	0.31	0.014
Main effects										
Material										
BCH		91	71	2.9	1.48b	0.14b	1.42	1.62	0.39	0.013
A-BCH-I		81	114	3.0	1.61a	0.19a	1.56	1.59	0.35	0.014
Dilution										
80:20		89	108	3.0b	1.55ab	0.17	1.47	1.61	0.41	0.010
40:60		94	125	3.8a	1.36b	0.18	1.60	1.69	0.38	0.017
0:100		76	46	2.0c	1.71a	0.16	1.41	1.52	0.32	0.014
Statistical significance										
Material		***	***	ns	*	*	ns	ns	ns	ns
Dilution		***	***	***	*	ns	ns	ns	ns	ns
M x D		***	***	ns	ns	ns	ns	ns	ns	ns

ns, *, *** indicate not significant, statistically significant differences at $P\leq0.05$, $P\leq0.001$, respectively. Values in the same column with different letters are statistically different at $P\leq0.05$ (Tukey test)

Table 8 Growth and leaf nutrient content (% dry weight) of seedlings of *Phillyrea angustifolia* grown in growth media produced by mixing raw biochar (BCH) or acidified biochar (A-BCH-II) with soil (experiment II)

Material	Dilution (% v:v)	Shoot length (cm)	Leaves plant^{-1}	Shoot dry mass (mg)	Root length (cm)	Root dry mass (mg)	N	P	K	Ca	Mg	Fe
BCH:Soil	30:70	55ab	80	2830ab	22	866ab	2.21	0.09	0.49	2.42	0.45	0.013
	20:80	49abc	86	2585b	21	830ab	2.03	0.09	0.45	2.40	0.41	0.011
	10:90	57a	83	3225a	19	964a	2.05	0.10	0.46	2.47	0.41	0.009
	0:100	46c	60	1711c	27	638bc	1.99	0.10	0.43	1.59	0.34	0.017
A-BCH-II:Soil	30:70	47bc	62	1925c	24	500c	2.22	0.09	0.39	2.88	0.44	0.012
	20:80	43c	76	1953c	22	501c	2.09	0.10	0.46	1.70	0.37	0.009
	10:90	43c	70	2008c	21	549c	1.87	0.12	0.53	2.10	0.35	0.023
	0:100	45c	60	1731c	26	634bc	2.00	0.10	0.41	1.60	0.35	0.016
Main effects												
Material												
BCH		52	77a	2590	22	824	2.07	0.10	0.46	2.22	0.40	0.013
A-BCH-II		45	67b	1902	24	547	2.05	0.10	0.45	2.07	0.38	0.015
Dilution												
30:70		51	71a	2378	23b	683	2.22	0.09	0.44	2.65a	0.45	0.013
20:80		46	81a	2269	21bc	666	2.06	0.10	0.46	2.05b	0.39	0.010
10:90		50	77a	2617	20c	757	1.96	0.11	0.50	2.28ab	0.38	0.016
0:100		45	60b	1721	27a	636	2.00	0.10	0.42	1.60c	0.35	0.017
Statistical significance												
Material		***	***	***	ns	***	ns	ns	ns	ns	ns	ns
Dilution		**	***	***	***	ns	ns	ns	ns	*	ns	ns
M x D		**	ns	***	ns	*	ns	ns	ns	ns	ns	ns

ns, *, **, *** indicate not significant, statistically significant differences at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Values in the same column with different letters are statistically different at $P \leq 0.05$ (Tukey test)

Figure 1

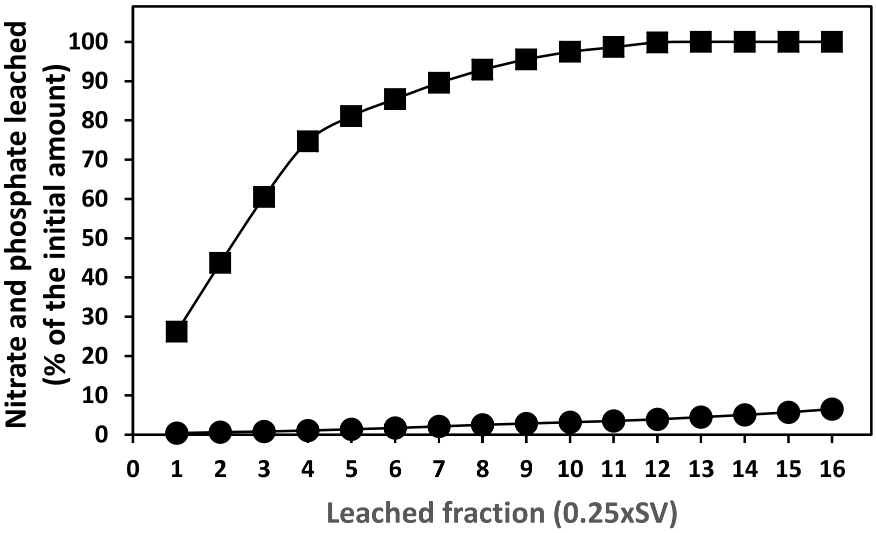

Figure 2

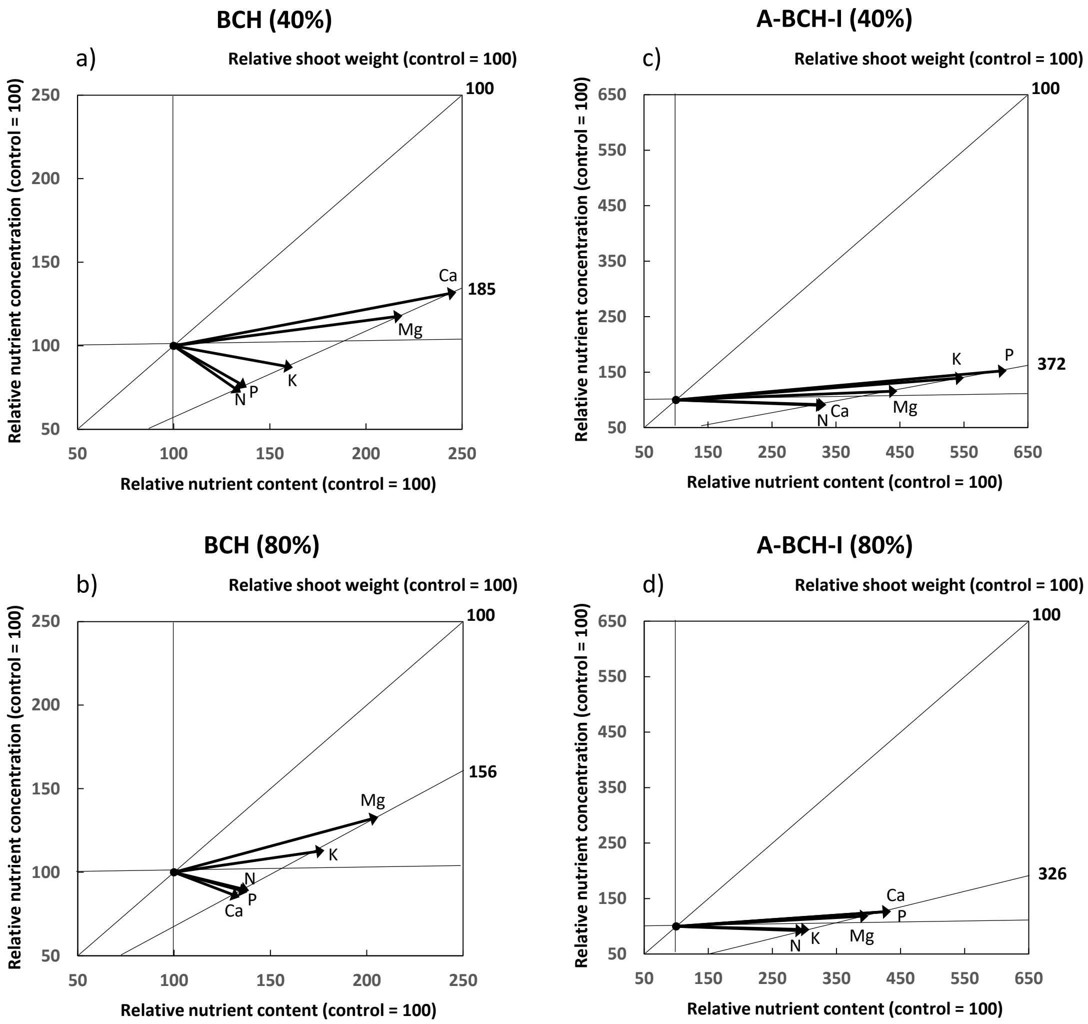

Figure 3

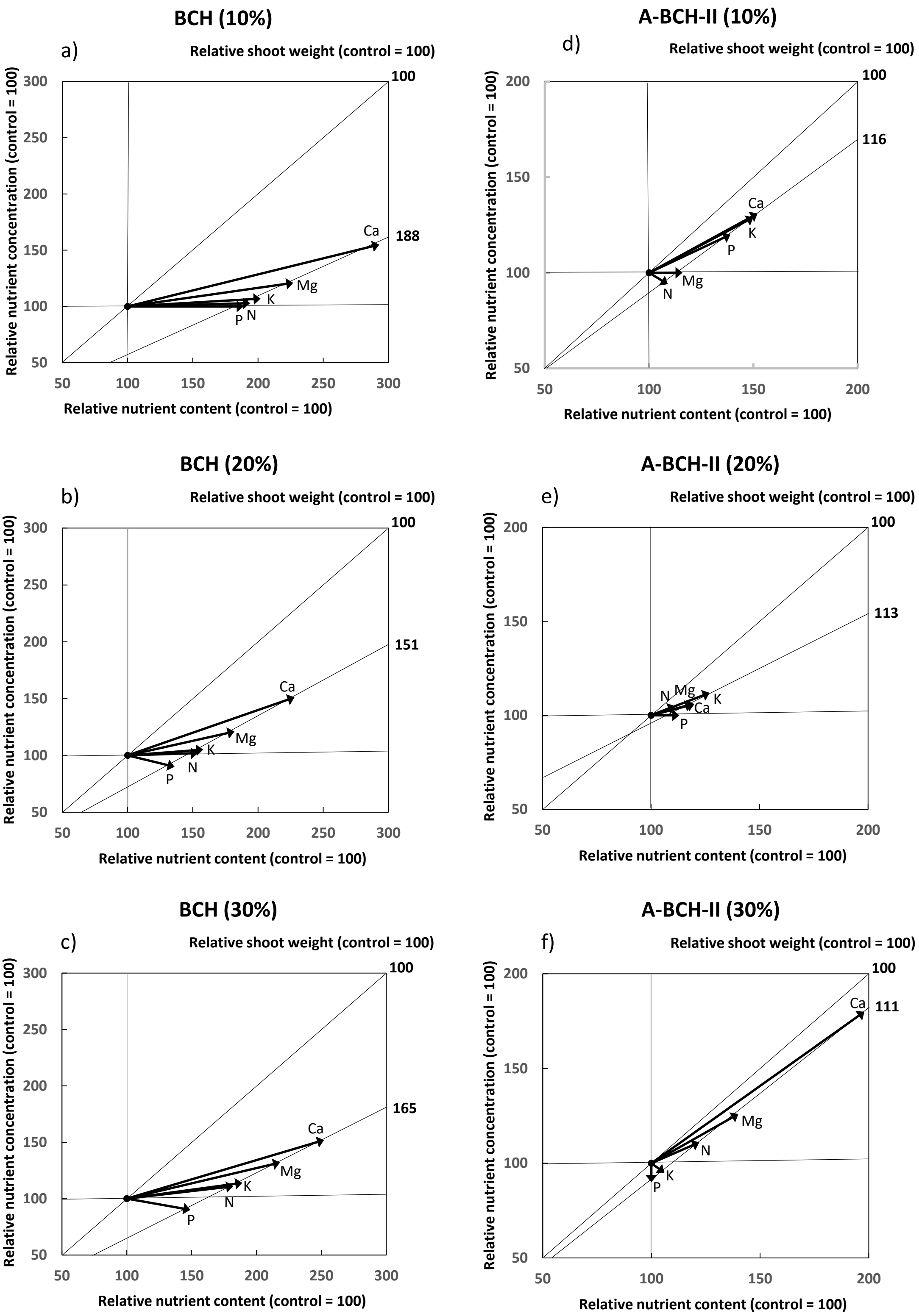

Fig. 1 Cumulative (% of the initial content) nitrate (■) and phosphate (●) leached from biochar acidified with 0.2M HNO_3 + 0.1M H_3PO_4. The experiment was carried out through the sequential pouring of 0.25 x SV (saturation volume = 467 mL L^{-1} BCH) fractions of water through columns filled with the biochar

Fig. 2 Relative response of macro-nutrient levels and shoot dry weight of *Rosmarinus officinalis* to the addition of BCH (a, b) or A-BCH-I (c, d) to peat-based growth media (experiment I). Control of 100% peat (closed circle) was normalized to 100.

Fig. 3 Relative response of macro-nutrient levels and shoot dry weight of *Phillyrea angustifolia* to the addition of BCH (a, b, c) or A-BCH-II (d, e, f) to soil-based growth media (experiment II). Control of 100% soil (closed circle) was normalized to 100.