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Biochar and vermicompost use as peat based growing media partial replacement to produce containerized ornamentals

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ESTUDIO DE SUSTITUCION PARCIAL DE MEDIO DE CULTIVO BASADO EN TURBA POR VERMICOMPOST Y BIOCHAR PARA LA PRODUCCION DE PLANTA ORNAMENTAL EN CONTENEDOR

BIOCHAR AND VERMICOMPOST USE AS PEAT BASED GROWING MEDIA PARTIAL REPLACEMENT TO PRODUCE CONTAINERIZED ORNAMENTALS

JOSE MARIA ALVAREZ DE LA PUENTE

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Memoria que presenta D. JOSE MARIA ALVAREZ DE LA PUENTE para optar al grado de Doctor por la Universidad de Huelva

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A mi familia

La satisfacción radica en el esfuerzo, no en el logro. El esfuerzo total es la victoria total.
(Mahatma Gandhi)

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Satisfaction lies in the effort, not in the achievement. Total effort is total victory. (Mahatma Gandhi)

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Abbreviations used: ANCOVA (analysis of covariance)**;** ANOVA (analysis of variance); As (air space); *B* (biochar); C (carbon); CAIC (consistent Akaike information criterion); CEC (cation exchange capacity); CO_2e (carbon dioxide equivalent); CT (cuticular transpiration); Db (bulk density); DI (damage index); EC (electrical conductivity); FIEL (freeze-induced electrolyte leakage test); GHG (greenhouse gas); HSD (Tukey-Honest significant difference); IM (inital substrate moisture content); LA (leaf area); LCA (life cycle assessment); P.h. (*Petunia hybrida*); P.p. (*Pelargonium peltatum*); Pt (total porosity); RC (relative conductivity); RGC (root growth capacity); RMP (recommended management practices); *S* (peat–based substrate); SDW (shoot dry weight); SE (standard error); SLA (specific leaf area); SOC (soil organic carbon); T (elapsed time); *V* (vermicompost); Va (container capacity); W (overall weight); WHC (water holding capacity); WT (water transpiration rate).

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INTRODUCTION

A biotic strategy to sequester carbon in the ornamental containerized bedding plant production: A review

Resumen

La identificación de opciones de mitigación del cambio climático es algo de interés para los investigadores a nivel mundial. Si bien se ha estudiado una amplia gama de técnicas para reducir las emisiones de gases de efecto invernadero (GEI) y el secuestro de carbono en cultivos y sistemas forestales, se ha investigado poco sobre cómo hacerlo en la horticultura ornamental. El sector industrial ornamental tiene algunos impactos negativos en el medio ambiente global, pero también presenta oportunidades para reducir las emisiones de GEI y aumentar el secuestro de C. Por lo tanto, el objetivo de este estudio de revisión fue sintetizar las posibles contribuciones de algunos sustratos utilizados en el sector hortícola en el secuestro de carbono. El enfoque específico de la revisión es el posible uso de compost, vermicompost y biochar como sustitutos de sustrato de cultivo para la producción de plantas ornamentales en contenedores. Alrededor de 11 millones de toneladas de turba *Sphagnum* se utilizan anualmente en el mundo para la producción hortícola. Por lo tanto, se evalúa en este trabajo el potencial de usar compost, vermicompost y biochar como medios de cultivo en base a datos de estudios de invernadero. El sustrato basado en turba se puede sustituir hasta un 30–35 % con compost o vermicompost y hasta un 20–25 % con biochar. Se incluyen algunos ejemplos de estudios de campo para realizar la evaluación del ciclo de vida del uso de estos medios de crecimiento. Una estimación del almacenamiento de C a largo plazo en el suelo indica que hasta 3 millones de toneladas de CO_2 equivalente podrían potencialmente almacenarse por año en el sector productivo global si los medios de cultivo basados en turba se sustituyen por compost / vermicompost y biochar en las proporciones mencionadas anteriormente. Finalmente, se discuten las sinergias entre compost, vermicompost y biochar cuando estos materiales se combinan como aditivos a medios de cultivo y, asimismo, se han identificado vacíos de investigación en esta área de actividad para futuras investigaciones.

Abstract

Identifying options of climate change mitigation is of global interest to researchers. Whereas wide range of techniques of reducing greenhouse gas (GHG) emissions and carbon sequestration have been studied in row crops and forest systems, little research has been done on the ornamental horticulture. The ornamental industrial sector has indeed some negative impacts on the global environment, but also presents opportunities to reduce GHG emissions and increase C sequestration. Thus the objective of this study was to synthesize the potential contributions of some substrates used in the horticultural sector to carbon sequestration. The specific focus of the review is on the possible use of compost, vermicompost and biochar as soilless substrate substitutes for containerized ornamental plants production. Around 11 million tonnes of sphagnum peat moss are used annually in the world for horticultural production. Therefore, the potential of using compost, vermicompost and biochar as growing media is assessed on the basis of data from greenhouse studies. Peat-based substrate can be substituted up to 30 % to 35 % by compost or vermicompost and up to 20 % to 25 % by biochar. Some examples from field studies are included to conduct the life cycle assessment of using these growth media. An estimate of C storage on the long-term basis in soil indicates up to 3 million tons of CO_2 equivalent as

the maximum C potential storage per year in the global productive sector if the peat-based growing media are substituted by compost/vermicompost and biochar at the ratios mentioned above. Finally, synergies between compost vermicompost and biochar are discussed when these materials are combined as growing media additives and research gaps in this area of activity have been identified for further research.

Background

Climate change and CO_2 sequestration

There is a concern in the scientific field about climate change and its present and future impacts on human wellbeing. An increase in the atmospheric concentration of CO_2 may increase the Earth's mean temperature and change the precipitation patterns (IPCC, 2014). Thus, there is a growing interest in identifying strategies of decreasing the amount of atmospheric CO_2 by reducing anthropogenic emissions (Lal, 2009). In the meanwhile, carbon (C) sequestration capacity of natural sinks (*i.e.*, oceans, forests, peat bogs) is also decreasing because of human activities (Raviv, 2015). The process of transfer and secure storage of atmospheric CO_2 into other long-lived C pools that would otherwise be emitted or remain in the atmosphere is called 'carbon sequestration' (Lal, 2008). Therefore, in this context, C sequestration may be a natural or an anthropogenically driven process. The objective of an anthropogenically driven C sequestration process is to balance the global C budget such that future economic growth is based on a 'C-neutral' strategy of no net gain in atmospheric C pool. Such a strategy would necessitate sequestering almost all anthropogenically generated CO_2 through safe, environmentally acceptable and stable techniques with low risks of leakage (Lal, 2008).

State of the art

Strategies to C sequestration

There are three main strategies of reducing CO_2 emissions to mitigate climate change: (i) reducing global energy use; (ii) developing low or no-C fuel sources; and (iii) sequestering CO_2 from point sources or atmosphere using natural and engineering techniques (Schrag, 2007). Regarding the last option, engineering techniques of CO_2 injection in deep ocean, geological strata, old coal mines and oil wells, and saline aquifers along with mineral carbonation of CO_2 constitute abiotic techniques. These techniques are expensive and prone to leakage. In comparison, biotic techniques are based on natural and cost-effective processes but have finite sink capacity (Lal, 2008).

Thus far, agriculture has been a major source of gaseous emission. Adoption of agricultural best management practices (*i.e.*, conservation agriculture, integrated nutrient management, precision agriculture, cover cropping, agro-forestry, micro-irrigation) can enhance resilience of soils and ecosystems against perturbations and also mitigate climate change. In this context, there are numerous land use and management practices, which must be discouraged. Notable among these are tropical deforestation, drainage of wetlands, cultivation of marginal/poor soils, intensive tillage, removal of crop residues, flood irrigation and biomass burning. Crop residues and animal dung must be used as soil amendments rather than as sources of household energy (Lal, 2013). Carbon sequestration in agricultural soils enhances sustainability of the land use systems. Increasing soil organic carbon (SOC) concentration in the root zone is beneficial in any situation to generate or maintain healthy soils (Lal, 2004a; Pardo *et al.*, 2017) and it also restores environmental quality and associated ecosystem services over the long time horizon (Lehmann, 2009). Carbon sequestration in ecosystems is measured by infrared gas analyzer to measure CO_2 eddy flux (Goulden *et al*., 1996). In soils, C sequestration is estimated by difference in biomass and soil carbon content over time (Lal, 2004a).

In this regard the “4 per Thousand” proposal at the 21st Conference of the Parties to the United Nations (UN) Framework Convention on Climate Change (COP21) in Paris on 2015, has called for a voluntary action plan to enhance SOC content of world soils to a 40 cm depth at the rate of 0.4 % per year. The strategy is to promote SOC sequestration through adoption of the above mentioned recommended management practices (RMPs) of C farming (Lal, 2016a). Thus, it is important to identify the specific plant cultures with a high capacity of C sequestration; however, the rate of SOC sequestration with adoption of RMPs may depend on soil texture and structure, rainfall, temperature, farming system, and soil management. (Lal, 2004b).

Substrates in ornamental horticulture

Much of the research towards reducing GHG emissions and C sequestration has been conducted in row crop and forest systems. In comparison, a limited research has been conducted on the specialty crop industry such as ornamental horticulture. The latter is an industry that impacts rural, suburban, and urban landscapes. Although this industry may have some negative impacts on the global environment (Nicese & Lazzerini, 2013), it also has opportunities to reduce GHG emissions and increase C sequestration (Marble *et al*., 2012). The horticultural industry was responsible for emitting 8.0 million tons of $C0_2$ in 1996. This was 12 % more than in 1989/90 (RSFGV, 1999), and has been growing since then. The ornamental horticulture global production reached a value of $37.1 billion in

2014. European Union (34.3 %), China (15.9 %) and USA (13.9 %) contributed 64 % of the economy (AIPH, 2017). In USA, five states (California, Florida, Michigan, North Carolina, and Ohio) accounted for 69 % of that value. Principal plant´s categories are annual bedding/garden plants 33.2 %, potted flowering plants 20.9 %, indoor/patio use 18.4 %, herbaceous perennial plants 14.8 %, propagative floriculture materials 0.9 %, cut flower 9.7 % and cut cultivated greens 2.1 %.The wholesale value for annual bedding and garden plants totalled $1.29 billion in 2015. This value represents 69 % of the total bedding and garden category. *Petunia sp, Geranium* sp., *Viola* sp., *Impatiens* sp. and *Begonia* sp. cultivars were the top five bedding plant crops grown in flats. These cultivars are usually grown in greenhouses. Initially, seeds/cuttings are cultivated in trays. Young seedlings are transplanted into containers/hanging baskets and grown to maturity (USDA-NASS, 2016).

Containerized plant production in horticulture primarily utilizes soilless substrates. In general, these substrates are primarily composed of organic materials such as peat moss and inorganic materials such as vermiculite and perlite (Bilderback *et al*., 2013). However, to date, little is known concerning the C sequestration potential of the horticulture industry as a whole; which is also critical to assessing its potential contribution to mitigating the climate change (Prior *et al*., 2011).

It is in this context that the review below is an attempt to synthesize the potential contributions of some substrates used in the horticultural sector to carbon sequestration. The specific focus of the review is on the possible use of compost, vermicompost and biochar as soilless substrate substitutes for containerized ornamental plants production.

Peat environmental concerns and peat substitutes

Nursery and greenhouse activities worldwide have been challenged to optimize their water and nutrients management (Majsztrik *et al.*, 2011). *Sphagnum* peat moss is the main substrate used in horticulture because of its homogeneous and ideal physical characteristics and high nutrient exchange capacity. As much as 10 to 11 Tg of this material may be used annually in the world for horticultural production (Interior, 2013). Globally, the total volume of materials used in growing media is difficult to estimate because recent data are not available for many areas of the world, including the Americas (both South and North), Australia, as well as Southeast Asia, where the process of growing out of soil has expanded in recent years but mainly into hydroponic systems in China, Japan, Thailand, and Malaysia (Carlile *et al.*, 2015).

Schmilewski (2017) reported that 34.6 Mm^3 of growing media were manufactured on 2013 in Europe, of which 93.8 % was organic materials. Peat was the predominant bulky ingredient (75.1 %), followed by organic constituents other than peat and compost (10.8 %) and then compost (7.9 %). An increase of 100 % in green compost utilized as growing media in EU occurred since 2005 (Schmilewski, 2009). Traditional peat extracting countries have a strong focus on peat but there is an ever increasing interest and trend to replace peat by using other organic materials including composts. Countries without indigenous peat resources, *i.e.* the Netherlands, Italy and Belgium, also strongly depend on peat as the main growing media constituent. The principal objective of using mineral materials in growing media is to fine-tune their physical properties, and not to replace peat. In countries like Germany, Austria and Italy with emphasis on recycling bio-waste as part of their circular economies, the use of composts in growing media has increased (~6 % between 2005 and 2013) (Schmilewski, 2009, 2017) and is likely to develop in other EU member states as targeted by the Circular Economy Strategy of the EU (EC, 2015).

In addition, environmental concerns questioning the peat use in horticulture are growing due to the number of environmental services provided by peatlands (Ostos *et al*., 2008). They include their habitat value, carbon sink function, regulation of the local water regime and quality and flood protection (Alexander *et al*., 2008). In fact, peat is no longer considered a renewable resource because it requires thousands of years (Hugron *et al*., 2013) to be able to generate. Although peatlands represent an important component of the global carbon cycle, storing 23 g m^{-2} y^{-1} of C (Waddington *et al*., 2002), that today means more than 600 Pg de C (Harenda *et al*., 2018), there are serious doubts about how current peatland will evolve under the climate change situation since these systems require very specific levels of moisture, temperature and insolation (Bragazza *et al*., 2016).

In any case, there is a consensus about the need to find alternatives to peat as growing media for horticulture in order to reduce the current exploitation and degradation of peatlands when they are in phase of extraction (Waddington *et al*., 2002). This point of view comes not only from the horticulture industry but also because the influence of macroeconomic issues based on the movements of consumers and decision-makers. Therefore, the challenge lies in identifying and using renewable materials with low costs of production and transportation (Gruda, 2011) and those having adequate physical-chemical characteristics. For instance in UK, environmental groups, government, and horticulture companies have organized themselves to recognize the environmental consequences of peat use in horticulture. In fact the industry is looking increasingly towards renewable raw materials such as green compost or processed timber by-products (Michel, 2010, Caron & Rochefort, 2013).

Composts appear to be a sound alternative to peat within growing media, in volumetric ratio anywhere between 30 to 50 % (even up to 100 % in specific cases), depending on their origin, composition, maturity and end use (Masaguer & López-Cuadrado, 2006); Raviv, 2013). Coco fibres may partly fulfil this role (Abad *et al.*, 2002). However, since the overall peat demand is growing on the market and the volume needed for peat replacement as a component of substrates greatly exceeds the availability of coco resources, replacement by coco will remain to be low. Moreover, it is expected that the price of coco is going to rapidly increase relative to other biomass in such situations (Caron & Rochefort, 2013). Therefore, the principle focus of this study has been on compost, vermicompost, and biochar, which are some of the industrial peat-based growing media substitutes (Carlile *et al.*, 2015).

Compost and vermicompost

Numerous studies have been undertaken to establish the potential substitution of peat with commercial compost and vermicompost, enhancing plant´s rooting and growth while also reducing the negative side effects (Garcia-Gomez *et al.*, 2002; Sardoei, 2014).

The UK was a pioneer in the research of compost as a substitute for peat (Prasad & Maher, 2001) due to the government decision to establish a deadline for the use of peat in horticulture, thus promoting research in this field (Sohi *et al.*, 2013). Compost from garden pruning and maintenance (green compost) was successful in that research and has since been widely used. Also compost of urban organic waste, bio-solids of sewage treatment plants together with green compost have been effectively tested as growing media in the industrial production of horticultural, forestry and ornamental seedlings (López *et al.*, 2005).

As composting technique has been expanding, each region/country has been testing the composting of its organic waste of silvo-agro industrial origin that has had more at hand. For instance, in Spain, the Lourizan Forestry Research Centre worked on composting of pine bark from sawmills (Miranda & Fernandez, 1992) to be used as growing media for forestry seedling. Later this bark-derived compost was used for the production of ornamental woody plants in container. In regions and countries where containerized ornamental production was important, this initiative was emulated by using organic materials from agro-industries. Such as in Valencia region (Spain) where an inventory of organic agro industrial by-products was carried out with the same goal of manufacturing substrates by composting aiming to utilize them in ornamental container production (Abad *et al*., 2001). Some of these raw materials were included cork powder (Carmona *et al*., 2003), two-phase olive oil mill waste (¨alperujo¨) (Fernández-Hernández *et al*., 2013), organic fraction of the guacamole industry (González-Fernández *et al*., 2015), organic wastes of greenhouse horticultural production (Mendoza-Hernández *et al*., 2014), citrus pulp (Gelsomino *et al*., 2010), grape marc (Trillas *et al*., 2006), brewery sludge (Sánchez-Monedero *et al*., 2004), etc.

In vermicompost, researchers used different manures for their transformation by means of lombriculture techniques to identify products that could be used in horticulture. So, mainly pig manure (Atiyeh *et al*., 2000; Arancon *et al*., 2005; Bachman & Metzger, 2008; Lazcano *et al*., 2009) and cattle manure (Tringovska & Dintcheva, 2012; Sultana *et al*., 2015) were used and also sometimes green and vegetable crop wastes (Fornes *et al*., 2012; Belda *et al*., 2013; Morales-Corts *et al*., 2014).

Peat based substrates were substituted at a 30-35 % average ratio by compost and vermicompost in the experiences mentioned in Table I.1. Both compost and vermicompost trials showed a beneficial effect related to substrate physical properties and different morphological parameters of the tested ornamental plants grown with these new materials. So, better growth (Do & Scherer, 2013; Mendoza-Hernández *et al*., 2014; Sultana *et al*., 2015) increases in shoot dry weight (SDW) (López *et al*., 2003; Belda *et al*., 2013; De Lucia *et al*., 2013) and root collar diameter (RCD) (Álvarez *et al*., 2001), better container capacity (CC) and water holding capacity (WHC) (Tyler *et al*., 1993) were recorded in different experiments where the peat-based substrate was partially replaced by compost or vermicompost.

The list presented in Table I.1 is not exhaustive and could be extended through other studies (Carrión *et al*., 2007) where for instance, disease suppressive microorganisms which have been extracted from compost are able to colonize the surface and roots of plants when applied properly (Al-Mughrabi *et al*., 2008).

Ansorena *et al*., (2014) also argued that it is necessary to consider the limitations that bio-waste compost presents as a component of substrates and as an organic fertilizer because of its high salinity and low N concentration. Another limiting property of the compost being used as substrate may be high alkalinity. To address the latter, elemental micronized sulphur is usually added to compost (Carrión *et al*., 2005, 2008). Also compost stability may be a key factor when compost is used as growing media to produce ornamental plants in container, so only mature compost should be utilized (Raviv, 2008, 2014).

Biochar

Biochar is another organic amendment that has the potential to be used as growing media additive and as peat substitute. Biochar is defined as a solid by-product obtained from the thermochemical conversion of biomass in an oxygen-limited environment. The process relies on capturing the off-gases from thermal decomposition of organic materials to produce heat, electricity, or biofuels (Lehmann, 2007).

'Terra preta do Indio' Amazonian soils, characterized by high levels of soil fertility, described by Sombroek (1966) started a worldwide interest to search how biochar would help to mitigate climate change (Laird, 2008; Woolf *et al.*, 2010; Montanarella & Lugato, 2013). Addition of biochar to soils can result, on average, in increased above ground productivity, crop yield, nutrient availability, microbial biomass and rhizobia nodulation among a broad range of pedo-climatic conditions. The limited number of case studies showing a negative effect of biochar on crop yield are consolidating the idea that biochar has either a null or positive effect on crop productivity (Souchie *et al.*, 2011; Alburquerque *et al.*, 2013; Biederman & Harpole, 2013; Carter *et al.*, 2013; Mulcahy *et al.*, 2013; Akhtar *et al.*, 2014; Thomazini *et al.*, 2015; Lima *et al.*, 2016; Olmo *et al.*, 2016).

In fact, the production of biochar from farm wastes and their application in farm soils offer multiple environmental and financial benefits (Srinivasarao *et al.*, 2013; Rivas, 2015).

The priming effect concept was initially introduced by Bingeman *et al.* (1953) and may happen when biochar is added to soil. If used to describe C turnover it means an added decomposition of organic C following an inclusion of easily decomposable organic materials to the soil (Dalenberg & Jager, 1989). In the present study, the most prominent interest is related to the negative result of the priming effect of biochar because a higher retention of carbon in the substrate. No study to this effect has been found when biochar was added to peat based horticultural growing media. Nevertheless, there are several references of biochar incorporation in soil causing a negative priming effect in sandy soils which may be the most easily assimilated into the peat-based horticultural substrates (Lu *et al.*, 2014; Keith *et al.*, 2015).

Biochar has also been considered as a possible peat replacement in horticulture (Peterson & Jackson, 2014). It has shown potential as replacement for aggregates like peat moss in growing media (Sohi *et al.*, 2013; Judd, 2016). Adding biochar to growing media can result in several benefits in terms of substrate quality. Biochar generally has a high cation exchange capacity (CEC) and a high nutrient holding capacity, thereby reducing nutrient leaching. Biochar can also be considered as a source of nutrients (nitrate-N, K, Fe, Mn, and Zn) (Nemati *et al.*, 2015). This property must be taken into consideration during nutrient management planning. Most biochars are alkaline and can neutralize the acidity of a peat-based substrate, hence reducing lime requirements (Zaccheo *et al.*, 2014; Bedussi *et al.*, 2015). However, the increase of pH following a biochar application in growing media limits its application as its affects growth in plant´s germination (Buss *et al.*, 2016). In general, biochar has a low bulk density and when incorporated into a growing mix helps to reduce the risk of substrate compaction and related problems (Nemati *et al.*, 2015). Biochar can affect both water retention (Cao *et al.*, 2014) and substrate´s aeration properties depending on its particle size distribution. The incorporation of fine-textured biochar in growing media promotes water retention properties (easy and total available water) (Nemati *et al.*, 2015). Biochar particle size distribution is affected by type of biomass and the pyrolysis temperature. Choosing a biochar with the right particle size distribution is important in producing a growing mix with the desired physical properties. High-temperature biochars can bind soil-C and other nutrients on a long-term basis. In addition, higher temperature biochars have higher surface area and more micropore volumes than those of lower temperature biochars (Mukherjee & Lal, 2013).

One of the main limiting factors to the use of biochar in the growing media industry is the production of black dust during handling. Increasing the initial water content of biochar or using pelleted biochar can overcome the dust issues (Dumroese *et al*., 2011).

It has also been reported in some phytopathological studies that biochar and its associated microorganisms have a suppressive effect on plant diseases similar to those possessed by the compost (Elad *et al*., 2010; Elmer & Pignatello, 2011; Kolton *et al.*, 2011; Zwart & Kim, 2012; Gravel *et al.*, 2013).

Several successful propagating ornamental plant experiments have been reported where peat and some other components were replaced by biochar (see Table I.2). The inclusion of biochar into substrates showed that plant´s quality and growth were similar to those from the standard peat substrates. Besides, some extra benefits were also observed in reducing nutrients and water loss, decreasing substrate bulk density, and creating a beneficial environment for microorganisms. In these experiments the peat-based substrate was substituted by biochar at a 20 to 25 % average ratio (Table I.2).

The wide range of raw materials to produce biochar include wood, bark and remains of coniferous (Zwart & Kim, 2012; Gravel *et al.*, 2013; Gu *et al.*, 2013; Fascella, 2015; Fascella *et al*., 2017; Dispenza *et al*., 2016) deciduous trees (Graber *et al*., 2010; Elmer & Pignatello, 2011; Northup, 2013; De Tender *et al*., 2016), agricultural (Dumroese *et al*., 2011; Sharkawi *et al*., 2014; Vaughn *et al*., 2015a; Kim *et al*., 2016) and gardening residues (Tian *et al*., 2012; Nieto *et al*., 2016) and biosolids (Méndez *et al*., 2016). The benefits derived from the addition of biochar included improvements of morphological parameters of plants growth but also those of the physical (Kaudal *et al*., 2015; Dumroese & Landis, 2016), chemical (Altland & Krause, 2012; Kaudal *et al*., 2015) and biological (Elmer & Pignatello, 2011) properties of the substrate and the resistance of plants to fungal infections (Elad *et al*., 2010; Zwart & Kim, 2012).

Carbon footprint reduction in containerized ornamental plants production

Several LCA (Life Cycle Assessment) studies have been conducted in different regions to determine which materials and activities contribute more to the GHG effect in ornamental horticulture. One of these studies assessed the material and energy inputs required to produce a *Petunia* × *hybrida* plant from initial propagation to delivery at a regional distribution centre. Impacts were expressed in terms of their contributions to the carbon footprint or global warming potential of a single finished plant in a 10-cm diameter container. Results showed that peat consumption represented 7.7 % of the overall CO_2e (Carbon Dioxide Equivalent) emissions (Koeser *et al*., 2014).

Two LCA studies conducted in Italy (De Lucia, 2013; Vecchietti *et al*., 2013) considered compost as growing media substitute. The use of different rates of sewage sludge compost in the preparation of growing media for potted *Bougainvillea* was evaluated to assess its efficiency for the replacement of peat and to quantify the environmental impact of such alternative substrates. The data from LCA showed that the addition of compost reduced the environmental impact of the plant nursery. Specifically, the use of compost reduced ODP (ozone layer depletion index) by 23-42 % and also the primary non-renewable energy consumption index by 40-80 % when compost was added to the mixture (as 25-70 % of compost inclusion respectively in both indexes).

Altieri & Nicholls (2012) and Martínez-Blanco *et al.* (2013) reported the positive effects of compost application as nutrient supply and carbon sequestration and also opined that the benefits were quantifiable, and tools for their consideration with LCA were available. Regarding the supply of plant nutrients, between 5 and 60 % of the N applied with compost was mineralized, depending on the time frame considered. Figures range between 35 and 100 % for P and between 75 and 100 % for K. Carbon sequestration rates have shown to be higher in the short term (up to 40 % of the applied C) and decreasing to 2–16 % over a 100-year period (Martínez-Blanco *et al.*, 2013). Hence, those benefits should be regularly included in LCA studies, although their quantification needs to be improved.

Russo *et al.*, 2008, in another LCA study on cyclamen in container production reported that as the peat is a non-recyclable organic material, it can find a substitute in the green composts obtained by the treatment of municipal garden green wastes and pruning wastes.

Finally, another study, conducted in Germany reported the amount of reduced GHG emissions by substitution of peat with biochar. This substitution could avoid emissions of up to 4.5 Mg of CO_2e by each Mg of peat substituted (2.8 Mg CO_2/Mg by biochar inclusion plus 1.7 Mg CO_2 Mg by peat substitution) (Steiner & Harttung, 2014).

In the studies and experiments mentioned above, peat based substrates were substituted at a 30-35 % average ratio by compost and vermicompost and 20-25 % by biochar. We have calculated reduced GHG emissions by considering these substitution ratios as well as average bulk density levels of peat based growing media, compost/vermicompost and biochar. We have taken into account that every year about 11 Tg of peat are consumed in horticulture. If 20 % of worldwide peat used in horticulture would be in containers production, about 3 Tg CO_2e will be the C potential storage per year that this container productive sector will be able to generate when peat based growing media has been substituted as above mentioned.

Research gaps

Globally, there is a lack of information about the total volume of materials used in growing media in countries with an important production in South and North America, Australia and Southeast Asia (Carlile, 2008; Schmilewski, 2017).

Research on how to use compost and vermicompost as partial replacement of peat based growing media to produce ornamental plants has been more addressed by research studies (Raviv *et al*., 1986; Edwards & Burrows, 1988; Carrión *et al*., 2007) than the use of biochar. There are also a number of research gaps about how to combine either compost or vermicompost with biochar to substitute peat in this ornamental horticulture industry. That is why we have tried below to identify potential research projects able to get answers to the pending questions.

Assuming that biochar is a panacea without strong scientific evidence and credible data, may aggravate controversies and dilemmas (Perry, 2011; Mukherjee & Lal, 2013; Lal, 2015). This is a key point considering biochar's characteristics variability due to raw materials and production systems (Lorenz & Lal, 2014). For instance, in some studies identical biochars produced different results with different plant species (Vaughn *et al*., 2015c). Some but not all biochars have been shown to improve water retention and increase overall plant growth in sand-based rooting media. Impact of biochar on improvement of water retention and increase overall plant growth in sand-based root zones may happen with some but not with all biochars (Vaughn *et al*., 2015b). Also, it would be necessary to identify from which tree species or type of waste material biochar would be most desirable for use in horticultural potting substrates (Vaughn *et al.*, 2015a).

Results from some biochar studies begin to provide evidence of mitigation strategies, which can be implemented in container plant production to help growers benefit from C offset programs, adapt to future legislation, and improve the environmental impact from container plant production without negatively affecting crop growth (Marble *et al.*, 2012). So, more product carbon footprint analyses are necessary to map out the climate impact in different horticultural production systems (Soode *et al.*, 2015). It would be also useful to know what CO_2 percentage could ornamental horticulture represent respect to global horticulture production.

Additionally, there are some experiments that demonstrate the synergy of combining biochar with compost in soil (Schmidt *et al.*, 2014). This positive association is caused mainly because the combination of both materials improved its fertility, not only in a short time span, but also on a medium and long term basis (Fischer & Glaser, 2012). Compost and vermicompost have shown a good synergy with biochar, but literature about this combination in ornamental horticulture is rather scanty. Just one study using vermicompost and biochar to produce ornamentals in containers was found (Alvarez *et al.*, 2017). Both materials were mixed with no prior composting. A complete set of 24 combinations, where a peat-based substrate was partially replaced by 0 to 50 % of dairy manure vermicompost and 0 to 12 % of biochar produced by pyrolysis of *Pinus monticola* wood at high temperature (600 to 800 ºC). Better *Petunia hybrida* and *Pelargonium peltatum* plant growth and flowering was obtained in some of the mixtures of biochar/vermicompost with no more than 30 % of vermicompost content than in the control group. Even if most plant responses are related to morphological parameters it would be interesting to also test physiological parameters as they may provide results regarding plants growth after transplanting into soil (Alvarez *et al.*, 2018).

There are some other studies where that kind of mix was applied to soil and assessed plant or soil responses (Schulz & Glaser, 2012; Ngo *et al*., 2013; Rodríguez-Vila *et al*., 2014). So, more experience combining compost or vermicompost with biochar to substitute peat-based substrates in ornamental horticulture should be promoted to learn whether their synergy would be interesting for the industry and with the objective of carbon sequestration. There are a number of publications where biochar was added to other organic materials to be co-composted or composted together and a synergy was evident during this combined process enhancing the final compost produced. Even if there is no evidence yet of the proven results when using this kind of final product to replace peat in ornamental production, these trends are briefly discussed herein because it would be pertinent to research this subject (Dias *et al*., 2010; Jindo *et al*., 2012, 2016; Schulz *et al*., 2013; Antonius *et al*., 2015; Barthod *et al*., 2016; Malińska *et al*., 2016).

The ornamental containerized plant sector needs to develop a better understanding of plant nutrient requirements, better technology to assess root zone conditions, and better fertilizers or practices that would be able to match ornamental plant nutrient requirements during the growing season in containers. With a satisfactorily resolution of this sector, Majsztrik *et al*. (2011) and Raviv (2013) concluded that horticulture can provide ecological services such as efficient and long-term carbon sequestration, while restoring soil fertility through the use of organic amendments. In this context evaluating how to include compost, vermicompost and biochar (and their mixes) may minimize leaching of nutrients from containers due to irrigation. This subject is also a researchable priority.

As Nemati *et al*. (2015) commented, compost, vermicompost and biochar are still not a standardized product, and its properties may differ depending on the source or the production process. The growing media industry cannot accept these variations and requires a high quality, homogenous, and consistent components. Therefore, it is important to launch a standardization program to certify those materials which meet quality standards for use in the growing media industry. In this sense, it is important to bridge the gap between research findings and commercial production of ornamental plants by assessing the experimental results at a commercial scale (Vaughn *et al*., 2015c; Derrien *et al*., 2016).

Economically, biochar has a greater potential to replace aggregates than peat in growing media mainly due to the high cost of these aggregates compared with that of the peat. Additional research is needed to evaluate the impact of biochar on growth and development of plants.

Conclusions

The use of organic materials as compost, vermicompost, and biochar as peat substitutes in the ornamental containerized bedding plant production, is an interesting biotic strategy to store carbon in garden soil. In the case of biochar the stored C could be maintained for centuries improving the life cycle analysis of this process.

Several studies have produced interesting results, but additional research is needed to evaluate those materials and how to combine them as compost-biochar or vermicompost-biochar which may produce similar or better plants while also similarly or better support the transplanting process.

Conclusiones

El uso de materiales orgánicos como compost, vermicompost y biochar usados como sustitutos de turba en la producción de plantas ornamentales en contenedor, es una estrategia biótica interesante para almacenar carbono en el suelo de los jardines. En el caso del biochar, la cantidad de C almacenado podría mantenerse durante siglos, mejorando el análisis del ciclo de vida de este proceso.

Varios estudios han producido resultados interesantes, pero se necesita investigación adicional para evaluar esos materiales y cómo combinarlos como compost-biochar o vermicompost-biochar de forma que puedan producir plantas similares o mejores y al mismo tiempo que respalden el proceso de trasplante también de manera similar o mejorada.

Tables

Table I.1. Growing media researches where compost and vermicompost have been used as substrate components.

Substitute type	Growing media	Raw material	% rate v/v	Plant species	Effects[a]	Reference
Compost	peat based substrate	organic fraction of urban waste	25	*Pelargonium, Salvia*	better growth	Do & Scherer, 2013
Compost	peat based substrate	sewage sludge, yard trimming and organic fraction of urban waste	25, 50	*Rosmarinus officinalis*	root collar diameter (8 to 10)% greater than control	López *et al.*, 2008
Compost	peat based substrate	sewage sludge and pruning rejects	55	*Bougainvillea*	60% increase SDW	De Lucia *et al.*, 2013
Compost	pine bark substrate	turkey litter	up to 16	*Cotoneaster dammeri*	increased (12 to 16)% CC and (17 to 30)% WHC	Tyler *et al.*, 1993
Compost	peat based substrate	green yard waste	20	*Solanum lycopersicum*	growth equal than control	Prasad & Maher, 2001
Compost	peat based substrate	nursery pruning	40	*Lantana camara, Rosmarinus officinalis*	higher overall quality	Russo *et al.*, 2016
Compost	peat based substrate	pruning from *Olea europaea*, *Pinus* sp. and *Picea* sp. and *Lolium perenne* clippings	20	*Lycopersicon esculentum, Cucumis melo, Lactuca sativa*	better growth	Ceglie *et al.*, 2015
Compost	peat based substrate	sludge, yard trimming and organic fraction of urban waste	20, 40	*Ceratonia siliqua, Olea europea, Quercus ilex*	RCD increased (23, 30 and 10)% respectively than control	Álvarez *et al.*, 2001
Compost	peat based substrate	sludge and urban waste	20, 40	*Pistacia lentiscus*	(509 to 730)% higher SDW than control	López *et al.*, 2003
Compost	peat based substrate	two-phase olive mill waste (71%) with olive leaves (29%) and urea (9 kg t^{-1})	25, 50	*Solanum lycopersicum, Citrullus lanatus*	better seed germination	Fernández-Hernández *et al.*, 2013
Compost	peat based substrate	sweet sorghum bagasse, pine bark and brewery slude	up to 67	*Brassica oleracea*	similar growth	Sánchez-Monedero *et al.*, 2004
Compost	peat based substrate	cow manure	10	*Solanum lycopersicum*	10% increase in roots volume	Lazcano *et al.*, 2009
Compost	peat based substrate	pruning waste	100	no plants	pH > 8, OM similar, CEC higher than control	Benito *et al.*, 2006
Compost	peat based substrate	crops waste sawdust and laying hen manure	25	*Solanum lycopersicum, Cucurbita pepo, Capsicum annuum*	better growth	Gavilanes-Terán *et al.*, 2016
Compost	peat based substrate	acacia pruning	45	*Lactuca sativa*	better growth	Brito *et al.*, 2015
Compost	peat based substrate	sewage sludge	30	*Brassica oleracea*	better growth	Perez-Murcia *et al.*, 2006

Compost	peat based substrate	cork, grape marc, olive marc and spent mushroom	100	*Cucumis sativus*	better resistance to damping-off	Trillas *et al.*, 2006
Compost	bark based substrate	organic fraction of urban waste	50	*Physocarpus opulifolius*	increased 60% SDW	Chong, 2005
vermicompost	peat based substrate	green and pruning wastes	30	*Petunia*	similar growth than control	Morales-Corts *et al.*, 2014
vermicompost	peat based substrate	pig manure	30, 40	*Calendula officinalis*	more vegetative growth and flowers	Arancon *et al.*, 2005
vermicompost	peat based substrate	chopped air-dried tomato-crop waste	75	*Calendula officinalis*	20% increase in SDW	Belda *et al.*, 2013
vermicompost	peat based substrate	pig slurry	100	*Solanum lycopersicum*	15% increase roots volume	Lazcano *et al.*, 2009
vermicompost	top soil	cattle manure	up to 10	*Passiflora edulis*	nursery commercial quality	Hidalgo *et al.*, 2009
vermicompost	peat based substrate	from tomato crop waste	50	*Rosmarinus officinalis*	better growth	Mendoza-Hernández *et al.*, 2014
vermicompost	dried sandy loam topsoil	cow manure	10	*Zinnia elegans*	better growth	Sultana *et al.*, 2015
vermicompost	peat based substrate	pig manure	20	*Solanum lycopersicum*, *Calendula officinalis*	better growth	Bachman & Metzger, 2008
vermicompost	peat based substrate	N/A	20	*Solanum lycopersicum*	similar emergence, growth and biomass allocation	Zaller, 2007
vermicompost	peat based substrate	pig manure	20	*Solanum lycopersicum*	increased 12.5% fruit weight	Atiyeh *et al.*, 2000
vermicompost	peat based substrate	cow manure	10	*Solanum lycopersicum*	60% increase in SDW	Tringovska & Dintcheva, 2012

[a] SDW: shoot dry weight; CC: container capacity; WHC: water holding capacity; RCD: root collar diameter

Table I.2. Growing media researches where biochar has been used as substrate components

Substitute type	Growing media	Raw material	% rate v/v	Plant specie	Effects^{a}	Reference
biochar	peat based substrate	*Pinus* sp wood	5, 10, 15, 20, 25 and 30	*Gomphrena 'Fireworks'*	similar growth as control	Gu *et al.*, 2013
biochar	peat based substrate	*Pinus* sp wood	5, 10, 20	*Acer rubrum, Quercus rubra*	alleviate disease progression and physiological stress caused by *Phytophthora* canker pathogens	Zwart & Kim, 2012
biochar	peat based substrate	*Abies alba, Larix decidua, Picea excels, Pinus nigra*	60	*Euphorbia* × *lomi*	better growth	Dispenza *et al*., 2016
biochar	peat based substrate	*Quercus ilex* wood	3% w/w	*Fragaria* × *ananassa*	160% increase in SDW	De Tender *et al*., 2016
biochar	peat based substrate	hardwood	20, 30, 40	*Calendula officinalis, Petunia* × *hybrida, Impatiens*	SDW similar or greater than control	Northup, 2013
biochar	peat based substrate	hardwood dust	10	*Asparagus*	increased arbuscular mycorrhizal root colonization	Elmer & Pignatello, 2011
biochar	peat based substrate	hardwood pellets and pelletized wheat straw	10,15	*Calendula officinalis*	increased plant height	Vaughn *et al.*, 2013
biochar	peat based substrate	*Abies balsamea, Picea glauca* and *Picea mariana* softwood bark	50	*Pelargonium hortorum*	similar growth as control	Gravel *et al.*, 2013
biochar	peat based substrate	crushed wooden boxes	25, 50, 75	*Helianthus annuus*	similar growth as control	Steiner & Harttung, 2014
biochar	peat based substrate	pruning residue	50, 75	*Lactuca sativa*	better growth as control	Nieto *et al.*, 2016
biochar	peat based substrate	green waste	50	*Calathea rotundifola* cv. Fasciata	22% total biomass increase	Tian *et al.*, 2012
biochar	peat based substrate	biomass	1, 5, 10	no plants	moderation of extreme fluctuations of nitrate levels	Altland & Locke, 2012
biochar	peat based substrate	agricultural or forestry residues	25	no plants	enhanced hydraulic conductivity and greater water availability	Dumroese *et al.*, 2011
biochar	peat based substrate	biosolids	10	*Lactuca sativa*	better growth as control	Méndez *et al.*, 2016
biochar +digestate	peat based substrate	wood pellets, pelletized wheat straw and field pennycress presscake + potato anaerobic digestate	25	*Solanum lycopersicum, Calendula officinalis*	increased growth of tomato plants and equal marigold as compared to control	Vaughn *et al.*, 2015a

biochar	peat based substrate	conifers wood	60	*Euphorbia* × *lomi*	higher stem diameter, leaves area, root length and number of flowers than control	Fascella, 2015
biochar	coco fiber	forestry and gardening waste	10	*Calendula officinalis, Petunia* × *hybrid*	better growth as control	Fornes *et al.*, 2013
biochar	coconut fiber and tuff	*Citrus* wood	5	*Capsicum annuum, Solanum lycopersicum*	better pepper growth and enhanced tomato plant height and leaf size.	Graber *et al.*, 2010
biochar	coconut fiber-tuff	*Citrus* wood	1, 3, 5% w/w	*Capsicum annuum, Solanum lycopersicum*	resistance against two foliar fungal pathogens (*B. cinerea* and *L. taurica*)	Elad *et al.*, 2010
biochar	coir peat	biosolids and greenwaste	up to 60	no plants	similar physical and chemical benefits than control	Kaudal *et al.*, 2016
biochar	coir peat+pine bark compost	biosolids and greenwaste	20, 40, 60	no plants	desirable physical properties such as high water holding capacity, low bulk density, air filled pore space and high surface area	Kaudal *et al.*, 2015
biochar	rice husk	rice husk	25	*Cucumis sativus*	better growth as control	Sharkawi *et al.*, 2014
biochar	coir dust, perlite and vermiculite	rice husk	5% w/w	*Brassica oleracea*	150% increase in SDW	Kim *et al.*, 2016

[a] SDW: shoot dry weight

Hypothesis and general objectives

This research aims to contrast the hypothesis that is possible to grow commercial quality plants of *Petunia hybrida* and *Pelargonium peltatum* using biochar as partial substitute of peat based growing media.

Those plants also will be able to adapt themselves conveniently to a garden soil after being transplanted.

Finally will contrast the hypothesis that is possible to diminish nutrients leachate when growing both species using biochar and vermicompost as peat based substrate partial substitute.

To contrast these hypothesis three different comparative greenhouse studies were conducted to assess the suitability of biochar (*B*) and vermicompost (*V*) as partial substitutes for peat-based growing media for ornamental plant production

First experiment was focused on determining if it was possible to grow containerized ornamental bedding plants (as petunia and geranium) with commercial quality using 24 different biochar / vermicompost mixes (CHAPTER 1).

In the second one, we selected from the first one the five best performing growing media and verified the physiological plant response when growing those species with our selected mixtures (CHAPTER 2).

Finally we checked containers leachates to verify if fewer nutrients were lost by irrigation when growing those species with our selected mixes (CHAPTER 3).

CHAPTER 1

Vermicompost and Biochar as growing media replacement for ornamental-plant production

Resumen

El vermicompost es un producto derivado de la degradación biológica acelerada de restos orgánicos realizada por lombrices de tierra y microrganismos. El biochar es un subproducto de la tecnología de pirolisis C-negativa para la producción de bioenergía a partir de materiales orgánicos. La producción de plantas en floricultura utiliza sobretodo sustratos basados en turba. Sin embargo, el drenaje de las turberas ha generado preocupaciones medioambientales, que han incentivado el interés en la investigación sobre productos complementarios que puedan incorporarse a los sustratos basados en turba. Por ello, se llevó a cabo un estudio comparativo en invernadero para evaluar la idoneidad del biochar (*B*) y del vermicompost (*V*) como sustitutos parciales de sustratos basados en turba para la producción de planta ornamental. Se compararon diferentes mezclas de *B*, en una proporción en volumen de 0, 4, 8, 12 %, y de *V* al 0, 10, 20, 30, 40, 50 %, con un sustrato basado en turba *(S),* usado como control, en el cultivo de geranio (*Pelargonium peltatum*) y petunia (*Petunia hybrida*). Los substratos fueron caracterizados según sus propiedades físicas y químicas y, asimismo, se evaluó el crecimiento de las plantas y la producción de flores. Las mezclas con niveles medio–bajos de *V* (10 – 30 %) y altos de *B* (8 – 12 %) en *Petunia* y *Pelargonium* generaron más crecimiento y mayor producción de flores que en el sustrato control. Los resultados obtenidos con diferentes proporciones de *B* y *V* son interesantes para reducir el consumo de turba en la producción de planta ornamental en contenedor y reducir la huella de carbono de este sector productivo comercial.

Abstract

Vermicompost is a product derived from the accelerated biological degradation of organic wastes by earthworms and microorganisms. Biochar is a by-product of the C-negative pyrolysis technology for bio-energy production from organic materials. Containerized plant production in floriculture primarily utilizes substrates such as peat moss. Environmental concerns about draining peat bogs have enhanced interests in research on complementary products that can be added to peat. Thus, a comparative greenhouse study was conducted to assess the suitability of biochar (*B*) and vermicompost (*V*) as partial substitutes for peat-based growing media for ornamental plant production. Different blends of *B* at a volume fraction of 0, 4, 8, 12 % and *V* at 0, 10, 20, 30, 40, 50 % were compared to a baseline peat substrate *(S)* as control in the cultivation of geranium (*Pelargonium peltatum*) and petunia (*Petunia hybrida*). Substrates were characterized for physical and chemical properties, plant growth, and flower production. Mixtures with low–medium *V* levels (10 – 30 %) and high *B* level (8 – 12 %) in *Petunia* and *Pelargonium* induced more growth and flower production than that of the control. These results obtained with different *B* and *V* associations are of interest to those who want to reduce peat consumption for the production of ornamental plants in containers and to reduce carbon footprint of this commercially productive sector.

Introduction

Research on biochar has used materials from diverse feedstock and applied to a range of mineral soils for numerous crops and farming systems. Understandably, results available in the literature are highly diverse and debatable (Jeffery et al., 2011; Lal, 2011)

Best results have generally been obtained when the recommended dosage of biochar was not greater than 10 to 15 % in volume (Beck et al., 2011) Studies about the effects of biochar blended with compost or vermicompost on substrates devoted to floriculture are scanty and not available. Therefore, the principal objective of this study is to analyze the effects of the vermicompost and biochar added in different ratios and compare to a commercially available peat-based substrate used for the production of petunia (*Petunia hybrida*) and geranium (*Pelargonium peltatum*), and how those ornamental plants will react in growth and flower production. The experiment is designed to test three hypothesis: *a)* vermicompost and biochar are good component partners to grow petunia and geranium in containers; *b)* it is possible to define a range of vermicompost and biochar proportions to produce commercial petunia and geranium plants; *c)* it is possible to maintain and/or improve the commercial production of these species while reducing the use of substrates from non-renewable sources. We have also considered in this work that it is possible to estimate how much C may be stored for long periods of time when growing *Petunia* and *Pelargonium* in a substrate where growing media has been substituted by vermicompost and biochar.

Material and methods

Organic substrates, plant material and experimental design

One type of vermicompost (*V*) and one type of biochar (*B*) were assayed in this study. The biochar was a commercial product called Soil Reef Pure 02 (Biochar Solutions Inc.) and produced by pyrolysis of *Pinus monticola* wood at high temperature (600 to 800 ºC). The vermicompost was also a commercial product from the Black Diamond

Vermicompost, and prepared by vermicomposting of dairy manure solids for 70 to 80 days which had been pre-composted for two weeks in an aerated composting system (Table 1.1, and Tables 1.A.1 to 1.A.3 in the appendix). Both materials were used as organic components to partially replace the normally used standard growing media by the Horticulture Department at the Ohio State University called Farfard 3B mixture by SunGro Horticulture Distribution Inc. (Tables 1.1, and 1.A.4 in the appendix). Such substrate is composed from the following ingredients: Canadian *Sphagnum* peat moss, processed pine bark, perlite, vermiculite, dolomitic limestone, and a wetting agent, being Peat:Bark:Perlite:Vermiculite volume ratio 6:4:2:1.

Two ornamental species were used in the experiments: *Petunia x hybrida cv*. Dreams Neon and *Pelargonium peltatum* cv. Summer Showers. The choice of cultivars was made based on their responses to substrate electrical conductivity (EC): tolerant for petunias (Mionk and Wiebe, 1961) and sensitive for geranium (Do & Scherer, 2013). Flower production of these two species of ornamental plants was studied because of their major commercial importance.

Treatments consisted of different mixtures of *V* and *B* with the commercially-available peat-based growing mix. Peat-based substrate in the tested mixes received a slow release fertilizer (Scotts Osmocote Plus 15-9-12 at 5.9 g L^{-1}). Twenty four treatments were prepared with the volume fractions detailed in Table 1.2. Taking into account this design, the separate effects of *V* and *B* could be also deduced by comparing separately the treatments containing $B = 0$ % on the one hand, and $V = 0$ % on the other hand, respectively.

Table 1.1: Biochar (*B*), vermicompost (*V*) and peat-based substrate *(S)* characterization. More details of properties of substrate components are shown in the appendix.(Results expressed in dry weight basis except other stated).

		Biochar	Vermicompost	Peat-based substrate
Organic Matter	(%)	91.6	72.7	55.3
Organic Carbon	(%)	75.8	35.0	n.a.
Total Nitrogen (N)	(%)	0.45	2.90	n.a.
Ammonia (NH_4-N)	(mg kg^{-1})	5.7	17.0	23
Nitrate (NO_3-N)	(mg kg^{-1})	64	3100	27
Sulfur (S)	(mg kg^{-1})	940	520	18
Sodium (Na)	(%)	0.520	0.300	0.002
Total Potassium (K)	(%)	20.0	0.54	0.01
Total Phosphorus (P)	(%)	0.370	0.436	0.001
EC (1:6 v/v fraction)	(mS m^{-1})	37.5	175	14.2
pH		9.5	6.5	5.47
Total Ash	(%)	8.4	27.3	44.7
Bulk density	(kg dm^{-3})	0.207	0.131	0.135

n.a.: not analysed.

Plastic containers (15.4 cm diameter, 800 cm^3), were filled with each of the mixtures and distributed in a random 5 blocks design for each of the two plant species (2 species x 24 treatments x 5 blocks = 240 containers).

Table 1.2: Notation used for the substrate mixtures (% in volume of each component): *S*, commercial peat-based growing media; *V*, vermicompost; and *B*, biochar.

Notation	Biochar (%)			
S:*V*:*B*	0	4	8	12
Vermicompost (%)				
0	100:00:00	96:00:04	92:00:08	88:00:12
10	90:10:00	86:10:04	82:10:08	78:10:12
20	80:20:00	76:20:04	72:20:08	68:20:12
30	70:30:00	66:30:04	62:30:08	58:30:12
40	60:40:00	56:40:04	52:40:08	48:40:12
50	50:50:00	46:50:04	42:50:08	38:50:12

Environment in the greenhouse

The experiment was conducted in the greenhouses of the Department of Horticulture and Crop Science at The Ohio State University, Columbus, OH. *Petunia* and *Pelargonium* plants were first produced in 200 plug trays (21.8 cm^3 per plug) for seed germination using a standard germination mix. Two *Petunia* and *Pelargonium* seeds per cell were sown in early February. After germination, just one seedling was kept. Trays were first placed in a germination glasshouse for 43 days at 23.7 °C and 54 % humidity. Seedlings were then transplanted into 15.4 cm diameter plastic containers and moved to a glasshouse (average temperature 20.1 °C and average humidity 29.3 %) during 8 weeks for *Petunia* and 11 weeks for *Pelargonium*. Standard propagation protocols for these species were followed. Plants were on benches, inside the greenhouse, and occupied 15 m^2 of surface. Within each block, plants were rotated periodically to minimize variation in microclimatic conditions. Seedlings in plug trays received irrigation by means of a micro sprinkler system and plants in container were watered manually as needed, based on environmental conditions and plant´s size under commercial usual conditions, moisture content was kept to field capacity. The entire growing period lasted for 124 days for *Pelargonium* and 90 days for *Petunia*.

Physical and chemical characterization of the substrates

Bulk density (*Db*), container capacity (*Va*), total porosity (*Pt*) and air space (*As*) were determined at the beginning of the experiment following the procedures for determining physical properties of horticultural substrates using the NCSU porometer (Fonteno and Bilderback, 1993). Organic matter was determined by dry ashing at 500 ºC. Fresh growing mix samples were used for the determination of soluble nutrients. EC and pH were determined using a 1 to 6 volume fraction aqueous extract (Ansorena Miner, 1994). pH was measured before filtration using a Accumet® Ap85 pH-meter. The filtrate was used for EC and mineral-content determinations after extract filtration. EC was determined with a conductimeter (Accumet® Ap85). Nitrate-N and ammonium-N contents were determined in the sample extracts by spectrophotometry in a flow autoanalyser (AA III, Bran + Luebbe, Norderstedt, Germany) (Ansorena Miner, 1994). Total element contents were determined in substrate components by ICP-OES after aqua regia digestion, and were expressed as total contents on a dry matter basis. In substrates, water soluble nutrients were determined by ICP-OES after extraction, and were expressed on a volume basis (Dahlquist and Knoll, 1978). Table 1.A.5 shows pH, EC and mineral nutrients contents of the different substrate mixtures at the beginning of the experiment.

Plant growth and flowering

At the end of the growth period, shoot dry weight (SDW) and number of flowers were recorded. In *Pelargonium* plants, the number of open inflorescences and inflorescence -buds were also counted. Shoot dry weight was obtained after oven-drying at 55 °C for 72 h. Chlorosis and spots in leaves were evaluated using a visual scale ranging from 1 to 10, being 1 a green plant with no chlorosis and no spots, and 10 a yellowish plant or a plant with more than 80 % surface covered by spots (Table 1.A.6).

Leaf nutrient concentration

Plant samples were ground to pass through a 0.5 mm sieve, and then digested by wet oxidation with high purity concentrated HNO_3 under pressure in a microwave oven (Miller, 1998). Mineral nutrients, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S), and trace elements iron (Fe), manganese (Mn), boron (B), copper (Cu), zinc (Zn) and sodium (Na), were determined by ICP-OES and expressed on a dry mass basis (Dahlquist and Knoll, 1978). Total nitrogen concentration was determined by spectrophotometry in a flow auto analyser after Kjeldahl digestion. Plant samples for quality control (WEPAL programs, Houba *et al*., 1996) were also analysed. Results obtained for these samples agreed ± 5 % with the certified results. Tables 1.A7, 1.A8.

Data analysis

One-way analysis of variance (SPSS Statistics 17.0) was carried out to determine statistically significant differences between treatments, being the treatment a fixed effect. Significant differences were established at $\alpha = 0.05$. To compare treatments, Duncan or T3-Dunnett tests were used in order to differentiate within homogeneous groups (according to variance homoscedasticity), and the Dunnett test was also used to compare each treatment with the control. In addition, a correlation and regression analysis were performed to establish the underlying relationships between treatments and measured parameters. A two-way ANOVA, with the main effects *V* and *B* and their interaction (*V* x *B*), was not carried out because *S* content greatly varied by varying *V* or *B*. Likewise, relevant tests of normality and homogeneity of variances were made before proceeding ANOVA, as well as transformation of the data if necessary.

Results

Physical and chemical characteristics of the substrates

The physical properties and OM of *Sphagnum* peat-based substrate (control) *S*, and the different mixtures with biochar (*B*) and vermicompost (*V*) studied are shown in Table 1.3. Although there are no universally accepted standards for the physical properties of container substrates, suggested guide ranges are outlined (Fonteno and Bilderback, 1993); (Yeager *et al*., 2000). *Db* and *Va* were always slightly above the recommended range, except for *Db* in the control treatment. *As* in some mixtures (76:20:04, 56:40:04, 72:20:08, 52:40:08, 48:40:12, 38:50:12) was slightly below the optimum range (6-13 %), and were not significantly different from each other.

Table 1.3: Selected physical properties and OM values of different substrate mixtures (treatments).

Treatment *S:V:B*[x]	*Db* (kg m^{-3})	*Va* (%)	*Pt* (%)	*As* (%)	*OM* (%)
100:00:00	135 a	70.1 a	81.0 abcde	10.0 e	55.3 a
96:00:04	137 ab	70.8 bc	80.5 abcd	9.8 de	60.7 b
92:00:08	147 bcdef	72.1 abcd	79.8 abcd	7.7 abcde	62.7 bc
88:00:12	146 bcde	72.4 abcde	80.0 abcd	7.6 abcde	66.3 bcde
90:10:00	141 abc	71.0 bc	81.1 abcde	10.1 e	60.6 b
86:10:04	144 abcd	71.0 abc	80.3 abcd	8.8 cde	64.0 bcd
82:10:08	159 ghijk	72.8 bcde	79.0 ab	6.0 abc	65.3 bcde
78:10:12	144 abcd	72.4 abcde	80.0 abcd	7.6 abcde	67.0 cdefg
80:20:00	149 cdefg	75.1 efgh	82.2 cde	7.2 abcde	69.3 efghi
76:20:04	150 cdefg	74.6 defgh	80.3 abcd	5.7 abc	69.3 efghi
72:20:08	163 ghik	73.2 bcdef	78.0 a	5.2 abcd	65.3 bcde
68:20:12	153 defghi	72.3 abcde	80.6 abcd	8.2 bcde	68.3 defgh
70:30:00	154 defghi	74.0 cdefg	81.2 abcde	7.2 abcde	67.0 cdefg
66:30:04	153 defgh	76.4 gh	83.9 e	7.5 abcde	69.3 defgh
62:30:08	156 efghij	73.5 bcdef	79.9 abcd	6.3 abcde	66.7 cdefg
58:30:12	164 ghik	74.8 defgh	81.9 bcde	7.0 abcde	66.7 cdefg
60:40:00	153 defghi	74.7 defgh	82.0 cde	7.3 abcde	69.0 defghi
56:40:04	158 ghijk	74.3 cdefg	79.3 abc	5.1 abc	70.0 efghi
52:40:08	164 hik	75.8 fgh	80.0 abcd	4.2 a	71.3 fghi
48:40:12	180 l	74.9 defgh	79.2 abc	4.4 ab	69.7 defgh
50:50:00	162 hijk	75.8 fgh	82.1 cde	6.2 abcde	71.7 ghi
46:50:04	155 efghij	73.4 bcdef	80.5 abcd	7.0 abcde	72.3 hi
42:50:08	164 ik	77.0 h	83.9 e	6.9 abcde	70.0 efghi
38:50:12	168 k	77.0 h	82.4 de	5.5 abc	73.7 i
p	***	***	***	***	***
Guide ranges[y]	100-300	45-65	78-88	6-13	

Db = Bulk density: *Va* = Container capacity: *Pt* = total porosity; *As* = air space;.*OM* = Organic matter.

[x] *S:V:B* , Volume fraction of peat based substrate (*S*), vermicompost (*V*) and biochar (*B*). Control treatment = 100:00:00

[y] Guide ranges (Fonteno & Bilderback, 1993; Harp *et al.*, 2011; Landis *et al.*, 1990; Yeager *et al.*, 2000).

p, significance level: *** indicates $p \leq 0.001$. Different letters in numerical columns indicate significant differences between treatments (Duncan test).

The general trend was a slight but significant decrease in *As* as *V* dose increased in the mixture ($p = 0.012$). Concentration of *V* was inversely related to *As* (r = -0.43, $p < 0.01$, n = 72), but positively to *Db* (r = 0.70, $p < 0.01$, n = 72) (Fig. 1.1) and *Va* (r = 0.70, $p < 0.01$, n = 72). Nevertheless, there was not significant relationship between *B* and these physical parameters *Db* (r = -0.15, $p < 0.01$, n = 72); *Va* (r = 0.11, $p < 0.01$, n = 72); Tp (r = -0.18, $p < 0.01$, n = 72). Treatments with $V \leq 10$ % and $B \leq 4$ % showed no significant differences in *Db* with the control treatment. The latter differed significantly ($p = 0.003$) from all other treatments containing $V \geq 20$ % regardless of the amount of *B* in the mixture.

Pt of the 24 treatments lay within guide ranges, and the control treatment did not differ significantly in *Pt* from other treatments.

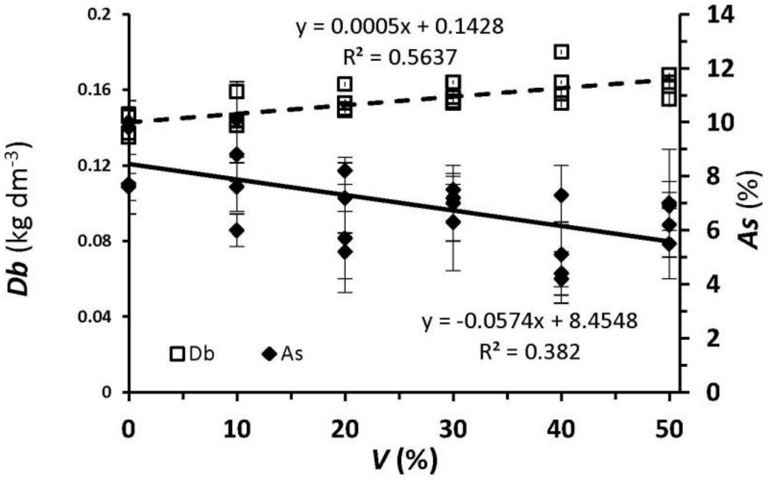


Figure 1.1: Relationships between vermicompost (*V*) content in the substrate and its bulk density (*Db*) and air space (*As*). For *Db* and *As* mean values (± SE) are shown (n = 24).

pH was slightly acidic (5.47) for commercial peat-based substrate and gradually increased (up to 6.57 at mixture 38:50:12) as vermicompost was added to the mixtures (Fig. 1.2, and Table 1.A.5 in the appendix). EC and pH were positively related to *V* ratio ($p < 0.01$, n = 24) (Fig. 1.2). However, pH and EC were not significantly related to *B*.

Concentration of N-NH_4^+ tended to decrease with higher doses of *B* for all levels of *V*, and concentration of N-NO_3^- increased (r = 0.97, $p < 0.001$, n = 24) with increasing rates of *V* (Fig. 1.3, and Table 1.A.5 in the appendix).

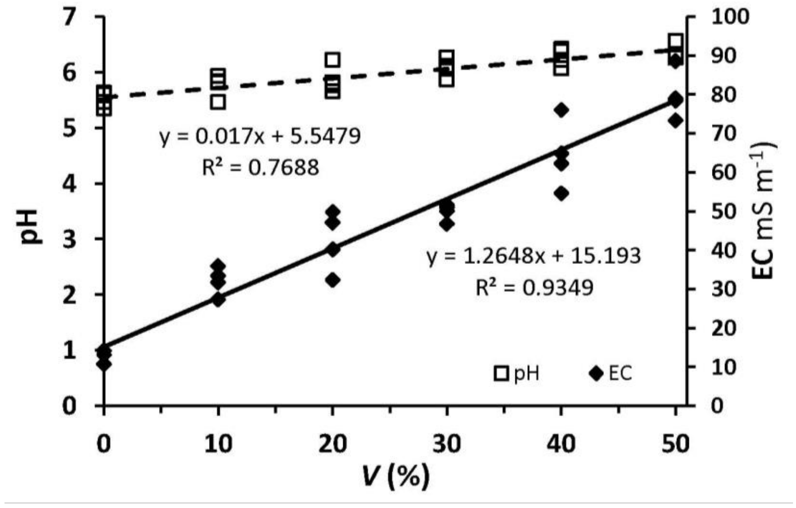


Figure 1.2: Relationships between vermicompost (*V*) content in the substrate and its pH and electrical conductivity (EC), (n = 24).

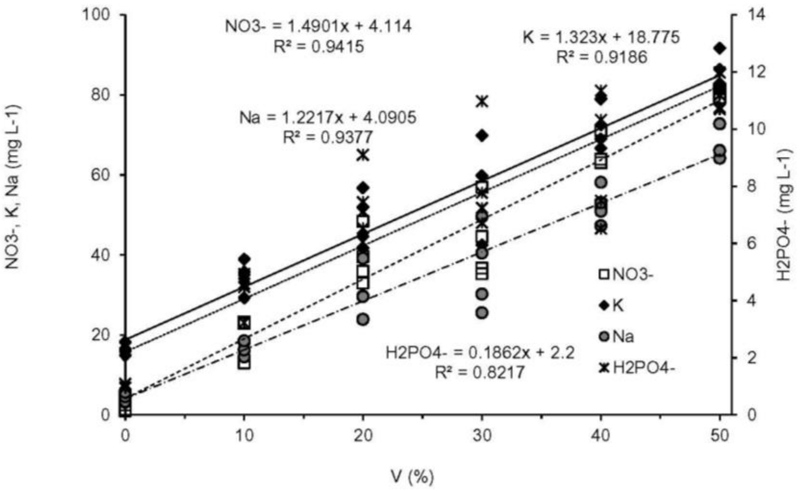


Figure 1.3: Relationships between vermicompost (*V*) content in the substrate mixture and nitrate (NO_3^-, dashed line), potassium (K, solid line), sodium (Na, dashed-dotted line) and phosphorus ($H_2PO_4^-$, dotted line).

Mixtures containing higher proportion of *B* and *V* had a higher organic matter content. Concentration of *OM* in all mixtures differed significantly from that in the control regardless of the amount of *V* and *B* in the mixture (Table 1.3).

Plant growth and flower production

Table 1.4 shows the biomass accumulated by the plants and the number of flowers per plant, for the two ornamental crops. In general, *B* rates of 4-12 % with moderate *V* proportions 10-30 % tended to produce the highest SDW for *Petunia*, but 50 % *V* resulted in a slight negative effect. The overall trend for *Pelargonium* indicated that 40–50 % *V* mixture did not favor the growth and flowering (Fig. 1.4). Chlorosis symptoms were observed only in *Petunia* in the case of the mixture 38:50:12 and they were not very marked. Chlorosis was not observed in *Pelargonium*.

Table 1.4: Plant-growth parameters of *Petunia* and *Pelargonium* grown on different substrate mixtures (treatments).

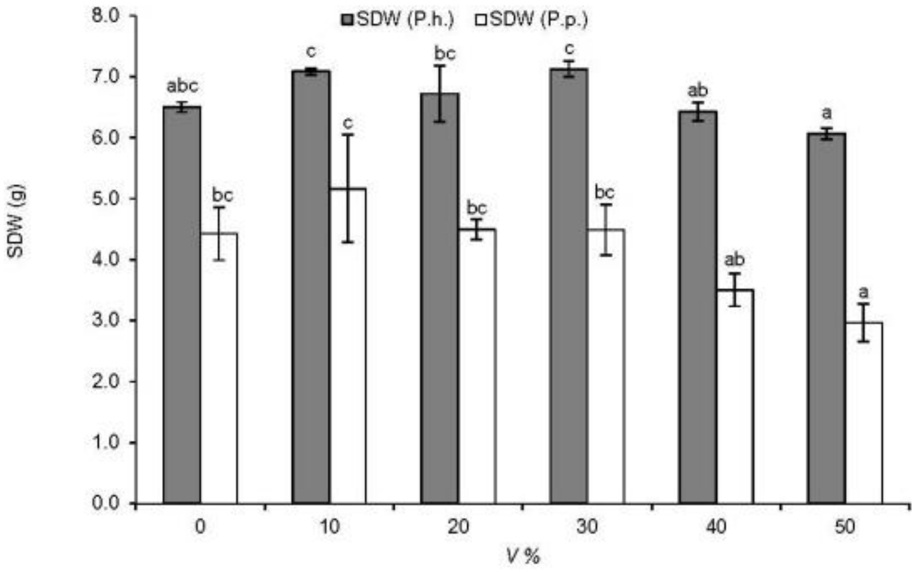
Treatments	Petunia			Pelargonium		
S:V:B^x	SDW (g)	Flowers (nº flowers)	Chlorosis (range)	SDW (g)	Flowers (flowers+buds)^y	Spots (range)
100:00:00	6.46 abcd	10.6 a	1.0	3.84 bcdef	0.77 bc	1.0
96:00:04	6.62 abcd	10.6 a	1.0	5.02 efg	0.91 bcd	1.2
92:00:08	6.64 abcd	11.8 ab	1.2	3.53 abcde	0.86 bcd	1.2
88:00:12	6.28 abcd	8.4 a	1.0	5.30 efg	0.97 bcd	1.6
90:10:00	6.94 abcde	9.6 a	1.0	5.20 fg	0.87 bcd	1.0
86:10:04	7.18 bcde	9.6 a	1.0	7.54 h	0.84 bc	1.2
82:10:08	7.12 cde	10.8 a	1.0	3.34 abcd	0.91 bcd	1.2
78:10:12	7.10 bcde	12.4 ab	1.0	4.56 bcdefg	1.06 cd	1.0
80:20:00	6.08 ab	9.0 a	1.0	4.54 defg	0.84 bc	1.2
76:20:04	6.14 abcd	9.2 a	1.0	4.64 defg	0.74 b	1.2
72:20:08	6.62 abcd	10.2 a	1.0	4.30 bcdefg	0.85 bc	1.0
68:20:12	8.04 e	17.0 b	1.0	4.50 cdefg	1.14 d	1.6
70:30:00	6.86 abcd	10.4 a	1.0	4.58 defg	1.01 bcd	1.2
66:30:04	7.4 de	8.4 a	1.0	5.60 fg	0.95 bcd	1.2
62:30:08	6.96 abcde	11.4 a	1.0	4.02 bcdef	0.83 bc	1.2
58:30:12	7.28 cde	13.0 ab	1.0	3.74 bcdef	0.90 bcd	1.6
60:40:00	6.82 abcd	10.8 a	1.0	3.80 bcdefg	0.90 bcd	1.0
56:40:04	6.178 abc	7.8 a	1.0	2.78 ab	0.35 a	1.0
52:40:08	6.18 abc	9.4 a	1.0	3.44 abcde	0.90 bcd	1.2
48:40:12	6.52 abcd	9.2 a	1.0	3.98 bcdefg	0.88 bcd	1.6
50:50:00	6.00 ab	8.8 a	1.0	3.54 abcde	0.73 b	1.6
46:50:04	6.14 abc	8.2 a	1.0	2.12 a	0.32 a	1.0
42:50:08	6.28 abcd	9.2 a	1.0	2.94 abc	0.78 bc	1.2
38:50:12	5.84 a	10.0 a	2.2	3.28abcd	0.82 bc	1.0
P	***	***	n.s	***	***	n.s

SDW: shoot dry weight.

^{x} *S:V:B* , Volume fraction of peat-based substrate *(S)*, vermicompost (*V*) and biochar (*B*). Control = 100:00:00

^{y} Transformed variable log 10

p, significance level: *** indicates $p \leq 0.001$. Different letters in numerical columns indicate significant differences between treatments (T3-Dunnett test). *n.s.*: not significant.



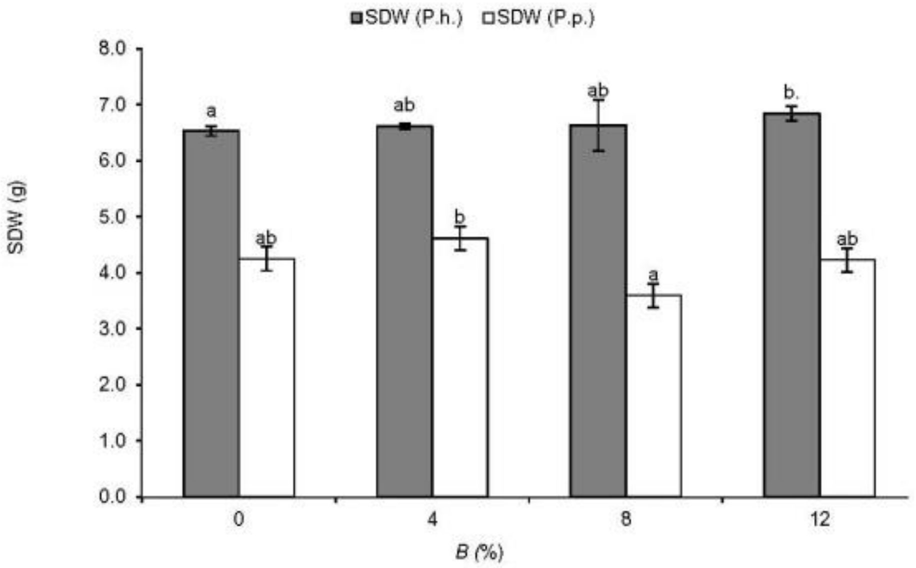


Figure 1.4: Mean values (± SE) of shoot dry weight (SDW) for *Petunia* (*P.h.*) and *Pelargonium* (*P.p.*) grown in different doses of vermicompost (*V*) and biochar (*B*) in the substrate. Significance level: p = 0.017 for *Petunia* and p = 0.044 for *Pelargonium*. Different letters indicate significant differences between *V* rates for every species.

For *Petunia*, it can be noted that leaf concentrations of Ca, K, Mg and Na were directly related to their availability in the substrate (r = 0.73, 0.89, 0.53 and 0.91 respectively, $p < 0.01$, n = 24). In *Pelargonium*, the leaf concentrations of Ca, K and Na were directly related to their availability in the substrate (r = 0.53, 0.50 and 0.95

respectively, $0.01 < p < 0.05$, n = 24), but an inverse correlation was observed between leaf Na concentration and SDW (r = -0.58, $p < 0.01$, n = 24). Additionally, in *Pelargonium*, inverse relationships were observed between SDW and available nutrient concentrations in the growth media: Ca (r = -0.57 $p < 0.01$, n = 24), K (r = -0.63, $p < 0.01$, n = 24), Mg (r = -0.55, $p < 0.01$, n = 24), Na (r = -0.64, $p < 0.01$, n = 24), N-NO_3^- (r = -0.63, $p < 0.01$, n = 24) and P (r = -0.54, $p < 0.01$, n = 24).

Discussion

Substrate characteristics

Substrates used in production of horticultural crops in containers are predominantly constituted by organic components and their physical properties are key factors to identify strategies that can be implemented to reduce negative effects on crop growth (Bilderback *et al.*, 2005). We found in this work that there was a trend to a slight decrease in *As* and an increase in *Db* with increasing *V* and *B* fractions. Being *V* and *B* more lightweight than *S*, it can be speculated that the substrates particles were filling the air gaps of the peat-based substrate. This resulted in a slightly less ideal substrate (Arancon et. al., 2005). However, considering mixtures containing $V \leq 30$ %, all of them were within the optimum range for *As*, while the deviation in *Db* was not very important in absolute value (Fonteno & Bilderback, 1993; Yeager *et al.*, 2000), taking into account that they were within the range of other nursery substrates like *Sphagnum* peat moss (0.06 to 0.12 kg dm^{-3}), other peat mosses (0.08 to 0.28 kg dm^{-3}), conifer barks (0.20 to 0.40 kg dm^{-3}), coconut fibers (0.18 to 0.20 kg dm^{-3}) or vermiculite (0.06 to 0.17 kg dm^{-3}) (Harp *et al.*, 2011; Landis *et al.*, 1990).

pH of the control substrate was slightly increased by *V*. These changes in pH coincided with those reported by Tyler *et al.*, (1993) according to which the pH increased in response to increasing concentrations of composted turkey litter added to a plant container medium. Ideal pH levels range for *Petunia* are from 5.5 to 6.2, and from 6.2 to 6.8 for *Pelargonium* (Irwin, 2002). With the exception of mixture 52:40:08 (with pH = 6.1), other mixtures (containing $V \geq 40$ %, or $B = 12$ % together with $V = 20$ % or 30 %) had pH values higher than 6.2, but chlorosis symptoms were not observed (except for 38:50:12 mixture with *Petunia*). Although pH was below 6.2 for some mixtures, chlorosis was not observed in *Pelargonium*. Therefore, based on the *Petunia* pH range, less than 40 % *V* should be used. Mixtures with $V \leq 10$ % might take a higher dose of *B* without exceeding the recommended pH limits for growing *Petunia*. Mixtures without *V* (i.e. $V = 0$ %) might take a higher dose of *B* without exceeding the recommended pH limits for growing *Pelargonium* and *Petunia*. The positive relationship between EC and *V* can be explained by the high EC of vermicompost. Similar results were reported by Atiyeh *et al.*, (2001). Klock, (1997) reported an increase in *EC* of 1.3 to 2.8 times over the control treatment with the addition of vermicompost. In the present study, EC increased 5.7 times over the peat-based substrate in mix 38:50:12.

Organic matter from the control was 55.3 % and gradually increased up to 73.7 % in substrate 38:50:12 because of the addition of vermicompost and biochar to the mixtures. In substrates containing $V \leq 10$ %, *OM* concentration was slightly more influenced by *B* content than by the *V* content.

Plant growth

For both species, SDW decreased for *V* ≥ 40 %, but to a greater extent for *Pelargonium* than for *Petunia*. This could be due to several reasons, such as increased EC and the decrease in *As*. *Pelargonium* was more affected by its higher sensibility to substrate salinity. Mixes with lower *As* (Milks *et al*., 1989) and higher pH and EC levels tended to induce lower SDW. Similar results were reported by Sultana *et al*., (2015) who observed that shoot height and total number of flowers of *Zinnia elegans* increased when grown in mixtures containing (10 – 20) % of vermicompost. On the other hand, in our work, *B* caused a lesser effect than *V* on substrate properties and on plant growth and nutrition, probably due to the lower amounts of *B* applied.

Overall, nutrient concentrations in the leaves were within the usual ranges suggested for these species (Mills *et al*., 1996), and did not show clear deficiency symptoms. The high Na leaf concentration in *Petunia* gives us an indication of its high salt tolerance, and the low Na leaf concentration in *Pelargonium* is typical of not tolerant species, because Na is not an essential nutrient for these plants and may be toxic (Hund-Rinke, 2008). The decrease in N, Fe and Mn for *Pelargonim* as *V* increased is characteristic when toxic levels of nutrients are present in growth media (Marschner, 2012), probably due to the effect of growth media salinity due to the dissolved mineral ions.

Environmental effect

Some studies have shown reductions in GHG emissions when *B* (Steiner & Harttung, 2014) is used as peat substitute for growing plants. *B* decomposes slowly (Kuzyakov *et al*., 2009) and can be stored for relatively long periods. *V* has a faster decomposition rate, so no significant C sequestration or storage in soil is expected by *V*, and this is why we only are going to calculate GHG emissions based in the biochar potential effect. Nevertheless, as peat volume substituted by *V* has a CO_2 sink role and, in addition, *V* contains mineral nutrients that potentially reduce the use of inorganic fertilizers contributing to reduce CO_2 emissions and energy consumption (Audsley *et al.*, 2003), *V* has been included in our calculation. Thus, this study is focused on the biochar effect to calculate how gaseous emissions associated with peat decomposition can at least be avoided if peat is substituted by *B*. The data presented herein shows that it is possible to grow *Petunia* and *Pelargonium* by replacing a portion of peat in a peat based substrate with a mixture of *V* and *B* at ranges up to 30 % *V* and 12 % *B*. It would be possible to save up to 117.8 kg of peat per tonne of substrate by substituting it with *V* and *B* taking into account bulk density of those materials (135, 206.9 and 131) kg m^{-3} for *P*, *B* and *V* respectively, and their weight to weight ratio in the mixture (47.7 %, 15.1 %, and 24.0 %, respectively). Thus, up to 151.4 kg of biochar and 239.6 kg of vermicompost may substitute 117.8 kg of peat in the new mixed substrate. The replacement of peat-moss with biochar could avoid up to 3.25 t of carbon dioxide equivalent (CO_2e) per tonne of peat substituted (Steiner & Harttung, 2014). Under the above mentioned assumption, the use of biochar could save up to 624.2 kg of CO_{2e} per tonne of the new substrate. Considering the mix 58:30:12 (*S*:*V*:*B*, volume basis) and its obtained *Db* measurement, it will be possible to store up to 88.74 gr of CO_{2e} per 800 ml container for long periods of time, first in the plant´s growing container and then in the soil after transplanting.(no C storage has been calculated when transplanting seedlings to containers because in seedling trays no peat substitution by vermicompost and biochar happened).

As shown in the present work, *V* and *B* can be mixed together in a substrate (hypothesis *a*). Both are renewable resources. *V* provides fertility and reduces inorganic mineral fertilization, and *B* contributes to carbon fixation in the long term. We have also partially verified hypothesis *b),* that an optimal range of *B* and *V* ratios will be obtained to grow these species. The top *V* rates (40 to 50 %) should not be reached as it was reported by García-Albarado *et al.*, (2010) and Sardoei, (2014). Nevertheless more research will be needed to verify how these species will grow with 0–30 % *V* mixes and higher ratios of *B* than 12 %. Finally, it is possible to state that hypothesis *c)* has been proven as a number of treatments produced plants of the same or better commercial quality than plants grown in the control peat-based treatment.

Conclusions

The data presented support the following conclusions:

- It is possible to grow containerized *Petunia hybrida* and *Pelargonium peltatum* plants with commercial quality after 3 or 4 months of cultivation, using substrates comprising a peat-based substrate mixed with biochar and/or vermicompost.

- As much as 30 % by volume of *V* and 12 % of *B* could be used in the substrate mixture without any adverse effects on plant growth and flower production. However, one must avoid adding the maximum doses of *V* (40 to 50 %) for growing *Pelargonium* and 50 % *V* for *Petunia*.

- Biochar and vermicompost offer great environmental advantages in their use as a peat-based growing media replacement in ornamental plant production because their C storage and / or CO_2 emission reduction.

The use of biochar and vermicompost is also compatible with the maintenance of the ornamental quality required for cultivated plants. Nevertheless more research would be necessary to a wider range of crops and with more standardized biochar and vermicompost products.

Conclusiones

Los datos presentados apoyan las siguientes conclusiones:

- Es posible cultivar plantas con calidad comercial de *Petunia hybrida* y *Pelargonium peltatum* en contenedor después de 3 o 4 meses de cultivo, utilizando sustratos compuestos por sustrato a base de turba mezclado con biochar y/o vermicompost.

- Podría usarse hasta el 30 % en volumen de *V* y el 12 % de *B* en la mezcla de sustrato sin ningún efecto adverso para el crecimiento de las plantas y la producción de flores. Sin embargo, se debe evitar agregar las dosis máximas de *V* (40 a 50 %) para el crecimiento de *Pelargonium* y 50 % *V* para *Petunia*.

- El biochar y el vermicompost ofrecen grandes ventajas ambientales en su uso como reemplazo de medios de cultivo a base de turba en la producción de plantas ornamentales debido a su almacenamiento de C y/o reducción de emisiones de CO_2.

El uso de biochar y vermicompost también es compatible con el mantenimiento de la calidad ornamental requerida para las plantas cultivadas. Sin embargo, sería necesaria más investigación para una gama más amplia de cultivos y con productos más estandarizados de biochar y vermicompost.

Tables (Supplementary material)

Table 1.A.1: Biochar (*B)* characterization (Soil Reef Pure 02 by Soil Control Lab). International Biochar Initiative (IBI) Level I.

	dry basis	unit	Method
Total Ash	8.4	%	ASTM D1762-84 (750c)
Organic Carbon	75.8	%	CHN by dry combustion
Inorganic Carbon	0.45	%	HCI treated
Hydrogen/Carbon (H:C)	0.48	molar ratio	
Hydrogen	3.0	%	CHN by dry combustion
Total Nitrogen	0.45	%	CHN by dry combustion
Total Oxygen	20.2	%	by difference
Liming (neut.value)	4.7	%CO_3Ca	Rayment & Higginson
Liming (carbonate.value)	3.8	%CO_3Ca	ASTM D4373
Activity (Butane)	7.6	g/100g	ASTM D5742 (butane)
Bulk density	206.9	kg m^{-3}	
Sulfur	0.094	%	
Energy (HHV)	27791	kJ/kg	
Moisture	12.7	%	ASTM D1762-84 (105c)

Particle Size Distribution ASTM D2862 granular		
(mm)	Retained (%)	Fraction (%)
> 19	0.0	0.0
16-19	0.0	0.0
9.5-16	0.0	0.0
6.3-9.5	0.0	0.0
4.0-6.3	0.4	0.4
2.0-4.0	23.0	22.5
1.0-2.0	53.9	31.0
0.425-1.0	86.8	32.9
< 0.425	100	13.2

Table 1.A.2 Element content in biochar (*B)* (Soil Reef Pure 02 by Soil Control Lab). International Biochar Initiative (IBI) Level II.

	dry basis	Unit	Method
AAs)	9.8	mg kg^{-1}	Bureau de Normalisation de Quebec
Cadmium (Cd)	0.17	mg kg^{-1}	(Amlinger *et al*., 2004)
Chromium (Cr)	28	mg kg^{-1}	(Amlinger *et al*., 2004)
Cobalt (Co)	4.6	mg kg^{-1}	Bureau de Normalisation de Quebec
Copper (Cu)	23	mg kg^{-1}	(Amlinger *et al*., 2004)
Lead (Pb)	12	mg kg^{-1}	(Amlinger *et al*., 2004)
Molybdenur (Mo)	< 0.2	mg kg^{-1}	Bureau de Normalisation de Quebec
Mercury (Hg)	< 0.2	mg kg^{-1}	(Amlinger *et al*., 2004)
Nickel (Ni)	17	mg kg^{-1}	(Amlinger *et al*., 2004)
Selenium (Se)	< 0.2	mg kg^{-1}	Bureau de Normalisation de Quebec
Zinc (Zn)	82	mg kg^{-1}	(Amlinger *et al*., 2004)
Boron (Bo)	117	mg kg^{-1}	(Council, 2002)
Chlorine (Cl)	1154	mg kg^{-1}	(Council, 2002)
Sodium (Na)	5194	mg kg^{-1}	(Council, 2002)
Potassium (K) Total	20	%	(Enders & Lehmann, 2012)
Phosphorus (P) Total	0.37	%	(Enders an& Lehmann, 2012)
Ammonia (NH_4-N)	5.7	mg kg^{-1}	(Rayment & Higginson, 1992)
Nitrate (NO_3-N)	64	mg kg^{-1}	(Rayment & Higginson, 1992)
Moisture	12.7	%	(Council, 2002)

Table 1.A.3: Vermicompost (*V*) characterization label information.

Component	dry basis	units	Component	Dry wt.	units
Total Nitrogen:	2.9	%	Lime as $CaCO_3$	4450	mg kg^{-1}
Ammonia (NH_4-N):	17	mg kg^{-1}	Organic Matter:	72.7	%
Nitrate (NO_3-N):	3100	mg/kg	Organic Carbon:	35.0	%
Org. Nitrogen (Org.-N):	2.6	%	Ash:	27.3	%
Phosphorus (as P_2O_5):	1.0	%	C/N Ratio	12	ratio
Potassium (as K_2O):	0.65	%	AgIndex	10	ratio
Calcium (Ca):	2.4	%	Copper (Cu):	170	mg kg^{-1}
Magnesium (Mg):	0.88	%	Iron (Fe):	5500	mg kg^{-1}
Sulfate (SO_4-S):	520	mg kg^{-1}	Lead (Pb):	2.3	mg kg^{-1}
Boron (Total B):	49	mg kg^{-1}	Manganese (Mn):	250	mg kg^{-1}
Moisture:	0	%	Mercury (Hg):	< 1.0	mg kg^{-1}
Sodium (Na):	0.30	%	Molybdenum (Mo):	4.2	mg kg^{-1}
Chloride (Cl):	0.16	%	Nickel (Ni):	27	mg kg^{-1}
pH Value:	NA	unit	Selenium (Se):	1.2	mg kg^{-1}
Bulk Density :	131.0	kg m^{-3}	Zinc (Zn):	910	mg kg^{-1}

Table 1.A.4: Standard peat based growing media (*S)* label information (mg kg^{-1}, except for pH).

Component	dry basis	Component	dry basis
pH	5.5-6.5	B	0.0-0.15
NH_4-N	0.0-50	Cu	0.0-0.12
NO_3-N	50-150	Fe	0.5-5.0
P	5.0-40	Mn	0.0-4.0
K	100-300	Mo	0.0-0.15
Ca	50-200	Na	20-50
Mg	40-200	S	100-250
Zn	0.0-1.0		

Table 1.A 5: Selected physico-chemical properties of different substrate mixtures (treatments). Units: mg L^{-1} for nutrients and mS m^{-1} for EC.

Treatment	pH	EC	N-NH$_4$	N-NO$_3$	H$_2$PO$_4$	K	Ca	Mg	SO$_4^{-2}$	Na	Fe
S:V:B[1]		(mS m^{-1})					(mg L^{-1})				
100:00:00	5.47	14.2	3.06	3.6	1.07	15.89	5.15	4.84	7.35	3.23	0.02
96:00:04	5.35	13.1	1.30	2.5	0.95	16.50	4.61	4.34	7.06	3.39	0.03
92:00:08	5.60	10.8	0.91	1.3	0.96	14.88	4.05	2.61	5.86	4.55	0.01
88:00:12	5.63	10.8	0.15	1.0	1.09	18.20	7.54	3.35	7.61	5.68	<0.01
90:10:00	5.46	31.7	0.18	23.0	4.47	29.29	13.13	11.60	7.13	14.54	<0.01
86:10:04	5.81	35.9	0.18	23.2	4.93	35.04	13.11	10.83	8.81	18.29	0.03
82:10:08	5.84	334	0.22	22.9	4.60	35.69	13.33	11.75	10.06	16.24	0.02
78:10:12	5.93	27.4	0.16	13.0	3.23	38.95	6.41	3.87	6.97	18.52	<0.01
80:20:00	5.76	47.1	0.16	39.6	7.45	44.59	19.74	15.33	8.04	29.74	0.01
76:20:04	5.82	32.4	0.13	48.4	9.10	56.76	23.30	18.28	9.90	39.13	0.02
72:20:08	5.66	40.2	0.24	33.0	7.12	41.80	16.66	13.77	8.51	23.88	0.01
68:20:12	6.22	49.8	0.17	35.9	6.53	51.98	15.14	12.66	8.96	29.60	0.01
70:30:00	5.87	46.8	0.19	36.7	7.24	42.48	18.56	15.67	8.26	25.57	0.02
66:30:04	6.06	50.0	0.41	35.3	6.72	42.45	21.98	16.46	10.97	30.25	0.02
62:30:08	6.11	51.6	0.14	56.9	10.98	69.81	28.06	21.44	13.19	49.55	0.01
58:30:12	6.27	51.1	0.06	44.6	7.77	59.81	21.96	14.31	9.72	40.49	0.01
60:40:00	6.42	76.0	0.22	63.0	6.52	72.53	24.25	17.10	10.74	50.91	0.02
56:40:04	6.22	54.6	0.32	63.9	10.32	69.02	26.75	20.35	10.54	53.03	0.02
52:40:08	6.07	64.9	0.14	71.1	11.34	78.99	29.55	23.00	12.69	58.15	0.01
48:40:12	6.38	62.3	0.09	52.4	7.49	66.68	20.37	15.41	8.15	47.29	0.01
50:50:00	6.28	88.6	0.25	81.5	11.98	82.79	36.31	26.61	11.83	66.14	0.03
46:50:04	6.26	79.0	0.23	79.1	10.76	82.22	31.99	24.28	11.90	64.16	0.02
42:50:08	6.31	78.5	0.09	79.3	10.70	86.41	31.81	23.55	12.22	66.06	0.01
38:50:12	6.57	73.3	0.06	81.5	11.2 0	91.66	36.60	25.83	16.81	72.78	0.01

[1] *S:V:B* , Volume fraction of peat based substrate (*S*), vermicompost (*V*) and biochar (*B*). Control: 100:00:00.

Table 1.A.6: Chlorosis level and spots ranges visually estimated in *Petunia* and *Pelargonium* leaves.

Code	Chlorosis level	Spots
1	No chlorosis green plant	No spots
2	Light chlorosis on terminal leaves	1-9 % leaf´s surface covered by spots
3	Medium chlorosis on terminal leaves	10-19% leaf´s surface covered by spots
4	Intense chlorosis on terminal leaves	20-29% leaf´s surface covered by spots
5	Light chlorosis on terminal leaves+ remaining leaves	30-39% leaf´s surface covered by spots
6	Medium chlorosis on terminal leaves+ remaining leaves	40-49% leaf´s surface covered by spots
7	Intense chlorosis on terminal leaves+ remaining leaves	50-59% leaf´s surface covered by spots
8	Very intense chlorosis on terminal leaves	60-69% leaf´s surface covered by spots
9	Very intense chlorosis on terminal leaves+ remaining leaves	70-79% leaf´s surface covered by spots
10	Yellowish plant	80-100% leaf´s surface covered by spots

Table 1.A-7: Leaf mineral concentrations (dry weight basis) of *Petunia* grown on different biochar and vermicompost mixtures.

Treatment	N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	Na
S:V:B[1]			(%)						(μg g^{-1})			
100:00:00	4.55	0.64	2.66	0.93	0.56	0.76	192.3	104.2	33.8	12.3	64.4	293
96:00:04	4.28	0.59	2.96	0.86	0.55	0.76	167.3	110.6	29.6	10.9	61.7	324
92:00:08	4.05	0.56	3.23	0.85	0.55	0.83	151.5	114.3	28.2	10.2	65.5	372
88:00:12	3.79	0.58	3.52	1.05	0.63	0.93	175.7	120.6	31.1	8.7	71.3	373
90:10:00	4.21	0.76	3.36	1.26	0.71	0.69	189.4	37.5	33.3	13.0	83.9	689
86:10:04	3.90	0.73	3.83	1.14	0.72	0.64	118.8	43.1	29.1	14.8	81.9	725
82:10:08	4.20	0.73	3.43	1.29	0.73	0.74	210.6	47.9	30.1	12.5	86.5	674
78:10:12	3.96	0.54	3.85	1.24	0.62	0.76	187.0	77.1	28.3	14.9	68.0	726
80:20:00	4.40	0.89	3.99	1.38	0.67	0.66	153.8	44.3	33.4	15.8	99.1	889
76:20:04	3.98	0.77	3.87	1.24	0.66	0.63	166.3	43.4	34.2	9.9	91.9	788
72:20:08	4.31	0.77	3.47	1.43	0.69	0.65	211.1	56.1	38.0	12.5	83.5	804
68:20:12	3.91	0.70	3.51	1.26	0.62	0.67	164.2	57.4	33.5	13.1	83.6	777
70:30:00	4.20	0.77	3.61	1.26	0.67	0.65	189.0	36.8	33.8	15.1	88.6	783
66:30:04	4.28	0.83	4.11	1.33	0.68	0.66	150.1	49.0	34.3	17.1	99.0	940
62:30:08	4.07	0.76	4.13	1.41	0.71	0.69	158.0	57.2	39.9	10.5	104.5	1080
58:30:12	4.03	0.72	4.11	1.38	0.65	0.70	135.4	62.1	33.3	11.9	104.0	1027
60:40:00	3.91	0.77	4.28	1.27	0.62	0.62	173.1	46.0	39.2	11.5	110.5	968
56:40:04	4.15	0.82	4.25	1.41	0.66	0.71	142.9	59.0	37.6	13.3	117.7	1058
52:40:08	4.18	0.78	4.10	1.42	0.66	0.67	169.1	66.1	33.7	16.6	108.0	1016
48:40:12	4.23	0.75	4.20	1.52	0.70	0.72	192.7	80.2	36.1	7.3	119.3	1193
50:50:00	3.98	0.81	4.55	1.29	0.64	0.65	175.6	50.9	35.3	9.2	118.3	1070
46:50:04	4.25	0.80	4.33	1.43	0.67	0.71	203.9	71.7	39.9	11.6	123.6	1130
42:50:08	4.18	0.80	4.38	1.48	0.68	0.70	178.1	77.4	36.5	13.2	135.1	1148
38:50:12	4.09	0.70	4.50	1.30	0.67	0.70	181.9	65.0	42.7	10.6	96.3	1086
Average	4.13	0.73	3.84	1.27	0.66	0.70	172.4	65.7	34.4	12.4	94.4	831
(SE)	(0.04)	(0.02)	(0.10)	(0.04)	(0.01)	(0.01)	(4.7)	(5.0)	(0.8)	0.5	(4.2)	(56)
Sug. Range[2]	3.85	0.47	3.13	1.20	0.36	0.33	84	44	18	3	33	3067
	7.60	0.93	6.68	2.81	1.37	0.80	168	177	43	19	85	10896

[1] *S:V:B* , Volume fraction of peat based substrate (*S*), vermicompost (*V*) and biochar (*B*). Control: 100:00:00

[2] Suggested ranges (Mills and Jones, 1996).

Table 1.A.8: Leaf mineral concentrations (dry weight basis) of *Pelargonium* grown on different biochar and vermicompost- based substrates.

Treatment	N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	Na
S:V:B[1]			(%)						(µg g^{-1})			
100:00:00	3.79	0.46	2.78	1.04	0.55	0.37	85.2	168.8	39.6	4.04	59.4	0.24
96:00:04	3.71	0.43	2.93	1.02	0.52	0.37	90.3	170.2	39.1	4.20	53.1	0.24
92:00:08	3.44	0.42	3.02	1.09	0.54	0.35	87.5	181.4	42.0	5.10	55.4	0.26
88:00:12	2.92	0.41	3.29	1.21	0.61	0.31	76.1	207.1	42.2	4.41	45.5	0.25
90:10:00	3.07	0.53	3.11	1.28	0.58	0.31	86.1	64.9	49.3	5.47	51.1	0.38
86:10:04	3.11	0.52	3.30	1.28	0.58	0.30	72.3	89.6	46.2	6.05	48.9	0.42
82:10:08	3.42	0.55	3.07	1.28	0.58	0.33	73.0	96.2	52.4	4.77	48.9	0.42
78:10:12	2.81	0.45	3.58	1.35	0.52	0.29	69.8	99.1	39.4	3.19	38.5	0.45
80:20:00	3.06	0.51	3.26	1.31	0.56	0.27	70.1	57.9	52.6	5.53	48.1	0.55
76:20:04	2.97	0.56	3.37	1.31	0.57	0.28	69.7	61.4	46.3	5.94	52.2	0.50
72:20:08	3.1	0.53	3.41	1.31	0.57	0.29	65.4	71.5	49.1	4.11	49.2	0.48
68:20:12	2.91	0.45	5.04	1.35	0.59	0.23	82.4	66.0	42.5	3.73	40.7	0.55
70:30:00	2.93	0.54	3.20	1.27	0.59	0.28	72.1	57.1	49.6	4.77	48.5	0.53
66:30:04	2.8	0.55	3.34	1.34	0.56	0.26	65.0	51.7	51.7	4.89	47.7	0.58
62:30:08	2.9	0.54	3.40	1.35	0.56	0.27	65.5	62.7	56.6	4.14	53.0	0.66
58:30:12	2.92	0.47	4.70	1.37	0.58	0.23	69.8	56.6	46.1	3.45	39.9	0.63
60:40:00	2.81	0.50	3.42	1.28	0.54	0.27	69.8	44.5	56.4	5.63	49.8	0.67
56:40:04	3.15	0.53	3.58	1.33	0.56	0.28	108.0	70.2	58.0	4.91	48.6	0.73
52:40:08	2.96	0.46	3.33	1.27	0.53	0.27	84.3	59.3	52.6	4.57	46.4	0.63
48:40:12	2.79	0.44	4.71	1.30	0.55	0.21	73.6	46.8	54.8	3.32	37.2	0.69
50:50:00	2.79	0.47	3.49	1.34	0.55	0.27	58.6	43.9	58.0	6.10	48.3	0.71
46:50:04	2.93	0.49	3.60	1.31	0.56	0.27	62.0	54.3	57.4	4.65	47.9	0.75
42:50:08	2.93	0.40	3.51	1.23	0.52	0.27	64.9	47.7	49.0	3.72	40.3	0.71
38:50:12	2.62	0.44	4.61	1.26	0.54	0.20	69.9	33.5	50.0	3.25	36.6	0.74
Average	3.04	0.49	3.54	1.27	0.56	0.28	74.6	81.8	49.2	4.58	47.3	0.53
(SE)	(0.06)	(0.01)	(0.12)	(0.02)	(0.01)	(0.01)	(2.3)	(10.0)	(1.3)	(0.18)	(1.2)	(0.03)
Sug. Range[2]	3.3	0.30	2.50	0.80	0.20	0.25	100	40	30	5	7	--
	4.8	1.24	6.26	2.40	0.51	0.70	580	325	75	25	100	

[1] *S:V:B* , Volume fraction of peat based substrate (*S*), vermicompost (*V*) and biochar (*B*). Control: 100:00:00

[2] Suggested ranges (Mills & Jones, 1996).

CHAPTER 2

Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture

Resumen

La turba de *Sphagnum* es el sustrato más utilizado en la producción de plantas en contenedor en floricultura. Sin embargo, el drenaje de las turberas debido a la extracción de turba ha aumentado la necesidad de buscar productos que puedan reemplazar la turba que se utiliza en la producción vegetal. Por ello, se realizó un estudio comparativo para evaluar el efecto de una mezcla de biochar (*B*) y vermicompost (*V*), como sustitución parcial de los sustratos basados en turba, sobre las características morfo-fisiológicas de plantas ornamentales. Se compararon diferentes mezclas de sustrato que contenían *B* y *V* con un sustrato control basado en turba (*S*) en el cultivo de dos especies de plantas ornamentales que se usan ampliamente en áreas urbanas: geranio (*Pelargonium peltatum*) y petunia (*Petunia hybrida*). Se evaluaron el crecimiento de las plantas y los parámetros fisiológicos. Los resultados mostraron que es posible cultivar plantas de contenedor de estas dos especies con calidad comercial, utilizando un sustrato a base de turba mezclado con biochar y/o vermicompost (hasta 30 % *V* y 12 % *B*). Las plantas en estos sustratos mostraron una respuesta fisiológica similar o mejor a las cultivadas en el sustrato control, un sustrato comercial a base de turba.

Abstract

Peat moss is the most used soilless substrate in the production of container plants in floriculture. Nevertheless, the drainage of peat bogs due to the peat extraction has increased the necessity of seeking products that could replace the peat that is used in plant production. Therefore, a comparative study was conducted to evaluate the effect of a biochar *(B)* - vermicompost *(V)* mixture, as a partial substitute for peat-based substrates, on the morpho-physiological characteristics of ornamental plants. Different blends containing *B* and *V* were compared to a baseline peat-based substrate (*S*) as control in the cultivation of two ornamental bedding plant species that are widely used in urban areas: geranium (*Pelargonium peltatum*) and petunia (*Petunia hybrida*). Plant growth and physiological parameters were assessed. Results showed that it is possible to grow container plants of these two species with commercial quality, using a peat-based substrate mixed with biochar and/or vermicompost (up to 30 % V and 12 % B). Plants in these substrates showed a similar or enhanced physiological response to those grown in the control using commercial peat-based substrate.

Introduction

Researchers have found that a good combination of biochar and compost is an acceptable growing media (Schmidt et al., 2014) because of the improvement of soil fertility over the short-, medium-, and long-term (Fisher and Glaser, 2012). Several residues have been used as sources of biochar included in growing media, such as biosolids (Méndez et al., 2016), urban wastes (Álvarez ML et al., 2017; Nieto et al., 2016) and deinking sludge (Méndez et al., 2015), among others. Vermicompost (from dairy manure) and biochar (from pine species) can be commonly found all around the world and their combination may play an interesting role in partially replacing peat as growing media (Alvarez JM *et al.*, 2017). Commercialization of ornamental plants involves not only morphological characteristics of plant quality (i.e. adequate size, dense foliage, leaf color, and number and color of flowers) but also enough vigor and capacity to maintain growth and withstand environmental stresses after leaving the nursery. Among traditional indicators of commercial plant quality parameters are those related to water stress resistance or low temperature tolerance, as well as the ability to continue growing after transplant (Landis *et al*., 2010; Santagostini *et al*., 2014), that are usually assessed at the end of the nursery growth period. Nevertheless, to our knowledge, there are few if any studies on the physiological responses of plants grown in a substrate composed of a peat-based growing medium and partially substituted by biochar and vermicompost.

Therefore, the main focus of the present study was to analyze: *1)* the usual morphological growth parameters such as Shoot Dry Weight (SDW) and number of flowers, *2)* some physiological traits related to plant response to environmental stresses, such as cuticular transpiration (i.e. the loss of water through the leaf epidermis when stomata are closed), *3)* whole plant transpiration, *4)* frost tolerance and *5)* root growth capacity. The latter two parameters are indicators of the general vigor of plants and their capacity to withstand several types of stress. The experiment was designed to test that there is no loss of physiological properties of two bedding plants when using a growing medium, whereby a non-renewable peat-based substrate is partially replaced by biochar and vermicompost.

Materials and methods

Experimental design and plant material.

A commercial peat-based growing mix (Farfard 3B mixture by SunGro® Horticulture Distribution Inc., Bellevue, WA, USA) was used as the control (*S*). Further, this commercial peat-based growing mix was partially replaced by biochar (*B*) and vermicompost (*V*) to make up the rest of substrate treatments. The peat-based substrate was comprised of Canadian *Sphagnum* peat moss, pine bark, perlite, vermiculite, dolomitic limestone, and a wetting agent, at 6:4:2:1 Peat:Bark:Perlite:Vermiculite volume ratio, and received a slow release fertilizer (Scotts Osmocote plus 15-3.9-10 N-P_2O_5-K_2O at 5.9 g/L).

The biochar and the vermicompost were also commercial products: Soil Reef Pure 02 (Biochar Solutions Inc., Carbondale, CO, USA) produced by pyrolysis of *Pinus monticola* wood at high temperature (600 to 800 ºC) in a downdraft gasifier-type reactor with 1 min residence time, and Black Diamond Vermicompost prepared by vermicomposting of dairy manure solids (which had been pre-composted for two weeks in an aerated composting system) for 70 to 80 days. More details of properties of substrate components are shown in chapter 1 and in Álvarez JM *et al.* (2017). Since *V* could increase substrate salinity, the two ornamental species used in this assay, *Petunia x hybrida* cv. Dreams Neon and *Pelargonium peltatum* cv. Summer Showers, were selected because they are bedding plants that are widely used in urban areas (Ignatieva *et al.*, 2009; Sendo *et al.*, 2010). They also have different salt tolerance. *Petunia* is more tolerant than *Pelargonium* (Mionk & Wiebe, 1958; Do & Scherer, 2013).

The control with the peat-based substrate (*S*) and six treatments per species containing different mixtures of *B* and *V* with the commercial peat-based substrate were selected. These treatments were chosen based on the plant size and flower production obtained in a previous experiment including an extended range of mixtures Álvarez JM *et al.* (2017), which suggested to replace *S* with *V* at a rate less than 30 %. As detailed in Table 2.1, at least three treatments were identical for petunia and geranium in this experiment (the control, and treatments 2 and 3 containing a slight and a moderate substrate replacement, respectively). The other three treatments had a slight difference in the *B* and *V* ratios.

Table 2.1. Volume fraction (%) of peat-based substrate (*S*), vermicompost (*V*) and biochar (*B*) used as substrate treatments (*S*:*V*:*B*). Control treatment was 100:00:00.

Treatment	***Petunia***	***Pelargonium***
1	100:00:00	100:00:00
2	86:10:04	86:10:04
3	68:20:12	68:20:12
4	82:10:08	88:00:12
5	78:10:12	70:30:00
6	58:30:12	66:30:04

Two hundred young seedlings were germinated in plastic plug trays (21.8 cm^3) in a glasshouse at 54 % average relative humidity and 24 °C average air temperature with a micro sprinkler irrigation system. Two sets of sixty seedlings were randomly selected from the plug tray and transplanted to 800 cm^3 plastic containers located on 8 m^2 surface benches in a greenhouse at 20 °C average air temperature and 29% average relative humidity (2 sets x 2 species x 6 treatments x 5 plants = 120 plants). Containers were watered manually as needed, based on environmental conditions and plant size under usual commercial conditions, and moisture content was kept to field capacity. The growing period was 20 weeks for *Petunia* and 24 weeks for *Pelargonium*. Plants were periodically moved to minimize deviations in microclimatic conditions.

Plant growth and physiological parameters

Due to the major commercial importance of these two species, plant size (evaluated through the shoot dry weight, SDW) and flower production were taken into account as morphological parameters in this assessment. SDW and number of flowers were evaluated at the end of the growth period, the number of flowers of *Pelargonium* plants being the open inflorescences plus inflorescence-buds. SDW was measured after oven-drying at 55 °C for 72 h.

As physiological parameters to be evaluated at the end of the nursery growth period, parameters related to mineral composition, to water conservation or consumption (cuticular transpiration –CT– and water transpiration by the whole plant –WT–, respectively), to root growth capacity (RGC) and to frost tolerance were chosen (Landis *et al.*, 2010).

Plant dry samples were crushed to pass through a 0.5 mm sieve, and digested by wet oxidation with high purity concentrated HNO_3 under pressure in a microwave oven (Miller, 1998). Nutrients (P, K, Ca, Mg. S), and trace elements (Fe, Mn, B, Cu, Zn, Na, Al), were determined by ICP-OES and expressed on a dry mass basis (Dahlquist and Knoll, 1978). After Kjeldahl digestion, spectrophotometry in a flow autoanalyzer was employed to determine total N concentration.

CT was assessed on one leaf per plant, five plants per treatment and species, using the method of Quisenberry *et al.* (1982). Hence, descending transpiration curves were constructed and used to calculate the CT (mmol m^{-2} s^{-1} of H_2O) by analyzing the rectilinear part of the curve of fresh weight *vs.* time. In addition, leaf area and leaf dry weight were measured in order to calculate specific leaf area (SLA, m^2 kg^{-1}). RGC was assessed according to Ritchie (1985). Five plants per treatment were transplanted with the root ball intact into larger containers (28.3 cm diameter, 1,260 cm^3 volume) filled with horticultural perlite of grade 2. Containers were placed on benches in a greenhouse with 22 °C average air temperature, 50 % average relative humidity and natural photoperiod (~12 h), and watered manually as needed. Eight weeks later, the perlite was carefully separated from the roots and the amount of new root growth was evaluated (i.e. new white roots emerged from the root ball). New roots were collected, cleaned, dried at 70 ºC until constant weight and weighed.

Frost tolerance was evaluated with a freeze-induced electrolyte leakage (FIEL) test. This test is based on the fact that freeze-damaged cell membranes leak electrolytes that can be measured with an electrical conductivity meter (Burr *et al.*, 2001). Several freezing temperatures were tested in advance and the freezing temperature that caused 50 % of leaf damage (i.e. -6.7 ºC) was selected for the test. This was assessed using the method described by Royo *et al.* (2003) on one fully developed leaf per plant. Therefore, the damage index (DI) was calculated at -6.7 ºC as: $DI_{6.7}$ (%) = 100 (RC - RCc)/(100 - RCc), with RC and RCc (relative conductivities) being calculated as follows: RC = 100*(EC_1-B1)/(EC_2-B2), RCc = 100*(EC_{1c}-B1)/(EC_{2c}-B2), where EC_1 and EC_2 were the initial and final, respectively, sample EC, and EC_{1c} and EC_{2c} were, respectively, the initial and final EC of the control (i.e. a sample which did not suffer the frost event). B1 and B2 were the EC of blanks included in the test. This damage index was an estimation of the amount of frost injury.

In addition, the water transpiration rate by the whole plant (WT, mmol m^{-2} s^{-1}) was measured in well-watered plants, taking into account the transpiring water during a full day, and calculated as follows: WT = (W1 - W2)/($LA \cdot T$), where W1 is the overall weight on the first day of the container, the substrate, and the plant (g), W2 is the overall weight on the following day (g), and both were measured just after dawn; then, the transpired water was calculated as W1 - W2 (g); *LA* was the leaf area of the whole plant (m^2); and *T* was the time elapsed between W1 and W2 (s). This was undertaken on three different days for every plant, in order to determine an average value per plant. To prevent water evaporation from the container surface to the air, the containers were wrapped with a white plastic bag.

Data analysis

One-way analysis of variance (SPSS Statistics 17.0) was carried out to determine statistically significant differences between treatments, being the treatment a fixed effect. Significant differences were established at $p = 0.05$. To compare treatments, Duncan or T3-Dunnett tests were used in order to differentiate within homogeneous groups (according to variance homoscedasticity), and the Dunnett test was also used to compare each treatment with the control. In addition, a correlation and regression analysis were performed to establish the underlying relationships between treatments and measured parameters. A two-way ANOVA, with the main effects *V* and *B* and their interaction (*V* x *B*), was not carried out because *S* content greatly varied by varying *V* or *B*. Likewise, relevant tests of normality and homogeneity of variances were made before proceeding ANOVA, as well as transformation of the data if necessary.

One-way analysis of variance (ANOVA, SPSS Statistics 17.0) was carried out for each species to determine statistically significant differences between treatments (at $\alpha = 0.05$), with the treatment being a fixed effect. The Tukey-Honest Significant Difference (HSD) or the Dunnett T3 tests were used to evaluate comparisons among the treatments and to differentiate within homogeneous groups.

For plant transpiration (WT), an analysis of covariance (ANCOVA) was used with two covariates for *Pelargonium* (leaf area, initial substrate humidity) and one covariate for *Petunia* (leaf area). The models were chosen for their accurate and lower goodness-of-fit indicator values of consistent Akaike information criterion (CAIC) (Table 2.2). As there was a liner relationship between substrate moisture content and daily water transpiration for *Pelargonium* ($R^2 = 0.314$, $p = 0.001$), it was decided to include the moisture content as a covariate for this species even though the CAIC was slightly lower for one covariate (leaf area) than for two covariates. In addition, correlation analysis between morpho-physiological parameters of plants was carried out.

Table 2.2. Model comparisons for daily plant transpiration (WT), being the full model performed by a fixed effect (substrate treatment [*Treat*]) and two covariates (leaf area [*LA*], and initial substrate moisture content [*IM*]). CAIC: consistent Akaike's information criterion. *p*: significant level for the fixed effect. The models selected are typed in bold.

Model effects	***Petunia***		***Pelargonium***	
	CAIC	*p (Treat)*	CAIC	*p (Treat)*
Treat (LA)(IM)	387.2	0.005	**328.8**	**<0.001**
Treat (LA)	**383.7**	**0.001**	326.1	<0.001
Treat (IM)	400.0	0.275	342.2	<0.001
Treat	395.6	0.250	340.6	<0.001

Results and discussion

Plant size and flower production

The biomass accumulated by the plants and the number of flowers per plant for the two ornamental crops grown in the different substrate treatments are shown in Figure 2.1. It can be highlighted that *Petunia* SDW and flower production were significantly lower in the control treatment compared with the other treatments ($p < 0.001$), except for flowers in 78:10:12 and 58:30:12. For instance, plant weight in treatment 86:10:04 was 115 % greater and produced 320 % more flowers than plant weight using the standard peat-based substrate.

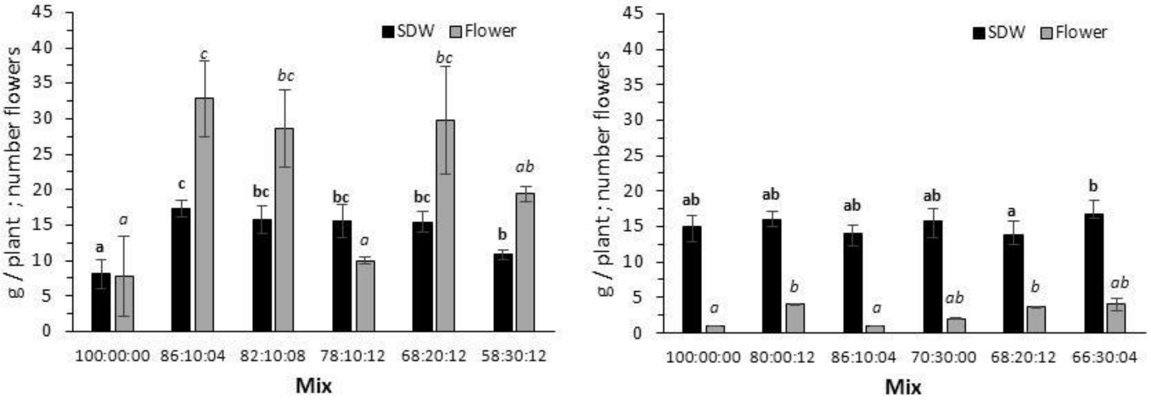


Figure 2.1. Shoot dry weight (SDW, g) and number of flowers of petunia (left) and geranium (right) grown in mixtures with different proportions of peat-based substrate (S), biochar (B) and vermicompost (V). Different letters show significant differences between substrates ($0.001 \leq p <\leq 0.0465$) (Tukey-HSD test for SDW both species, and Flowers in *Petunia*; Dunnett T3 test for Flowers in *Pelargonium*).

The improvement of *Petunia* SDW and *Petunia* and *Pelargonium* flowering are interesting results that should allow growers to substitute peat-based substrate by using V and B. These favorable results were obtained when $B \leq 12$ % and $V \leq 30$ % volume fraction were used. To our knowledge, no similar results have been found in container production of ornamental plants. There are studies in which peat-based substrates were partially replaced by biochar in horticulture for the production of vegetables (Mulcahy *et* *al.*, 2013) or ornamentals (Tian *et al.*, 2012) with good results, but without incorporating both materials *V* and *B* combined as partial substitution of a peat-based substrate. *B* and *V* can complement each other since *V* provides nutrients, and *B* increases cation-exchange capacity and C fixation in the long-term (Fisher and Glaser, 2012; Alburquerque *et al.*, 2013; Mukherjee & Lal, 2013).

Physiological parameters

Plant transpiration rate (WT) in *Petunia* was significantly (p = 0.001) lower in the control treatment than in the other treatments for well-watered plants (Figure 2.2).

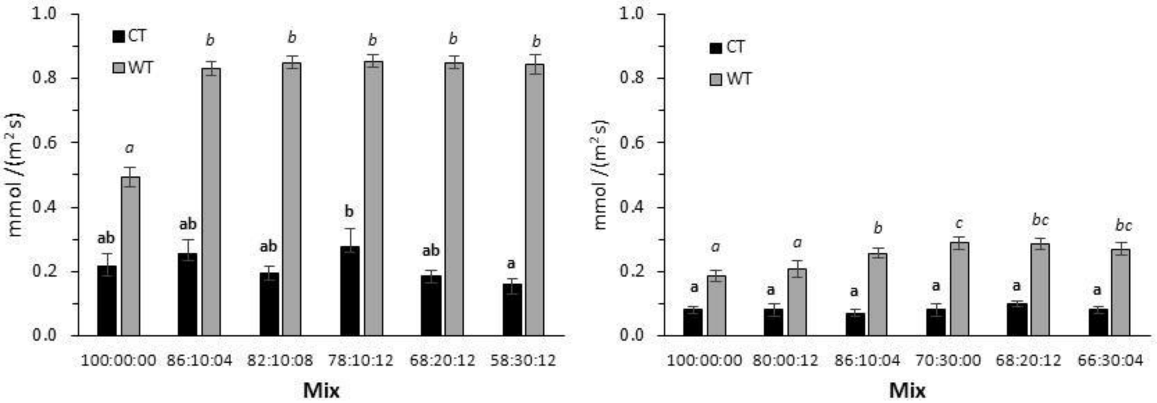


Figure 2.2. Cuticular transpiration (CT, mmol/(m^2s)) and plant transpiration rate (WT, mmol/(m^2s)) for well-watered plants of petunia (left) and geranium (right) grown in mixtures with different proportions of peat-based substrate (*S*), biochar (*B*) and vermicompost (*V*). Letters show significant differences between substrates ($0.001 \leq p < 0.0225$) (Tukey-HSD test for CT both species, and WT in *Pelargonium*; Dunnett T3 test for WT in *Petunia*). CT in *Pelargonium* was not significantly different among substrates (p = 0.703).

However, *Pelargonium* control plants significantly (p <0.001) transpired less than mixtures 86:10:04, 70:30:00, 66:30:04 and 68:20:12 (Figure 2.2). Hence, the *Petunia* plants in the control treatment, under well-watered conditions, saved more water than in mixtures containing *B* and *V*, but at the same time growth and flower production decreased. Only substrates containing less than 14% of the organic amendments (*B* + *V*) in *Pelargonium* showed a lower water loss. Therefore, although the addition of *V* and *B* led the plants to consume more water than the control plants, the greater physiological activity could have boosted growth and flower production. This fact was highly evident for *Petunia*.

Differences in cuticular transpiration (CT) among the control treatment and the mixes were not significant, hence this physiological response due to the inclusion of *V* and *B* in the substrate mixture was not detrimental to plants, and the water loss when the stoma are closed (i.e. leaf permeability) varied minimally (Villar-Salvador *et al.*, 1999). In other words, in the event that the plants suffer from a short period of water stress, plants grown on the new substrates will not decrease their capacity to conserve water.

Table 2.3. Root Growth Capacity (RGC) of petunia and geranium grown in mixtures with different proportions of peat-based substrate (*S*), biochar (*B*) and vermicompost (*V*). Different letters within the same column show significant differences between substrates (Tukey-HSD test).

Petunia		***Pelargonium***	
Treatment *S:V:B*	**RGC** (g)	**Treatment** *S:V:B*	**RGC** (g)
100:00:00	0.15 ± 0.02 a	100:00:00	0.67 ± 0.03 ab
86:10:04	0.20 ± 0.01 ab	86:10:04	0.59 ± 0.05 ab
68:20:12	0.22 ± 0.03 ab	68:20:12	0.60 ± 0.01 ab
82:10:08	0.18 ± 0.04 ab	88:00:12	0.82 ± 0.12 b
78:10:12	0.26 ± 0.03 b	70:30:00	0.50 ± 0.05 a
58:30:12	0.19 ± 0.03 ab	66:30:04	0.52 ± 0.01 a
Average ± SE	0.2 0± 0.01	Average ± SE	0.63 ± 0.04
p	0.025		0.031

With respect to *Petunia* RGC, the results were slightly better in every treatment than the results in the control, but no significate differences were observed except for the mixture 78:10:12 (Table 2.3).

Pelargonium control plants did not differ in RGC from other mixtures. Consequently, after transplanting, root growth is expected to be similar in plants cultivated in a peat-based substrate than in plants where *V* and *B* were incorporated into the substrate in different proportions. Hence, the general physiological plant state has not been altered. To our knowledge, there are no related results in the ornamental horticultural production in container in the existing body of literature.

Regarding the freeze damage index ($DI_{6.7}$), mean values were 56.0 ± 7.5 % for petunia and 83.3 ± 6.2 % for geranium, without significant differences among treatments ($p > 0.05$). This means that plants showed a similar response in any treatment, as in the research results of Birchler *et al.* (2001) with Douglas-Fir seedlings. Therefore, the addition of *V* and *B* maintained plant frost resistance in spite of increasing plant size and inflorescence production (i.e. increasing growth and metabolic activity).

Table 2.4. Leaf mineral concentrations (dry weight basis) of *Petunia* grown on different substrate mixtures.

Treatment	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Na**	**Fe**	**Mn**	**B**	**Cu**	**Zn**
S:V:B[1]			(%)							($\mu g\ g^{-1}$)		
100:00:00	2.13	0.43	3.85	1.76	0.44	0.44	0.43	85.73	68.8	14.9	9.81	62.8
86:10:04	2.06	0.45	3.44	1.63	0.53	0.51	0.59	97.36	46.0	14.1	10.84	73.4
68:20:12	1.92	0.48	3.34	1.67	0.50	0.47	0.52	74.39	48.9	14.7	8.15	70.5
82:10:08	1.93	0.49	3.46	1.60	0.46	0.47	0.55	79.54	56.7	13.0	9.45	75.1
78:10:12	2.06	0.45	3.34	1.66	0.49	0.47	0.52	64.56	57.2	14.7	8.11	66.8
58:30:12	1.98	0.53	3.86	1.69	0.53	0.47	0.50	76.08	44.1	15.5	11.23	82.9
Average	2.01	0.47	3.55	1.67	0.49	0.47	0.52	79.61	53.6	14.5	9.60	71.8
(SE)	(0.04)	(0.04)	(0.42)	(0.14)	(0.05)	(0.04)	(0.09)	(16.90)	(10.7)	(1.9)	(2.32)	(8.4)
Sug. Range[2]	3.85	0.47	3.13	1.20	0.36	0.33	0.31	84	44	18	3	33
	7.60	0.93	6.68	2.81	1.37	0.80	1.09	168	177	43	19	85

[1] *S:V:B* , Volume fraction of peat-based substrate (*S*), vermicompost (*V*) and biochar (*B*). Control, 100:00:00.
[2] Suggested ranges (Mills & Jones, 1996).

Overall, nutrient concentrations in leaves were within the normal ranges suggested for these species (Mills & Jones, 1996), and did not manifest clear deficiency symptoms

(Tables 2.4 and 2.5), although slightly lower N and Fe concentrations were obtained for both species.

Table 2.5. Leaf mineral concentrations (dry weight basis) of *Pelargonium* grown on different substrate mixtures.

Treatment	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**Na**	**Fe**	**Mn**	**B**	**Cu**	**Zn**
S:V:B[1]			(%)							(μg g^{-1})		
100:00:00	1.48	0.25	2.39	1.57	0.67	0.17	0.40	77.7	252.2	27.3	4.96	43.8
86:10:04	1.52	0.36	2.62	1.62	0.61	0.18	0.41	80.2	162.8	29.8	4.84	48.6
68:20:12	1.55	0.41	3.07	1.51	0.56	0.17	0.49	72.5	89.2	31.7	4.50	38.2
88:00:12	1.49	0.26	2.59	1.60	0.68	0.18	0.37	78.0	266.2	27.4	4.06	36.0
70:30:00	1.54	0.42	3.03	1.53	0.58	0.18	0.48	64.8	86.0	32.9	4.90	41.2
66:30:04	1.39	0.44	3.35	1.60	0.56	0.17	0.53	80.4	92.5	32.4	5.01	46.9
Average	1.49	0.35	2.84	1.58	0.61	0.18	0.45	75.5	158.1	30.2	4.71	42.4
(SE)	(0.04)	(0.01)	(0.07)	(0.02)	(0.01)	(0.01)	(0.01)	(7.21)	(15.4)	(3.7)	(1.00)	(1.3)
Sug. Range[2]	3.3	0.30	2.50	0.80	0.20	0.25	--	100	40	30	5	7
	4.8	1.24	6.26	2.40	0.51	0.70		580	325	75	25	100

[1] *S:V:B* , Volume fraction of peat-based substrate (*S*), vermicompost (*V*) and biochar (*B*). Control, 100:00:00.
[2] Suggested ranges (Mills and Jones, 1996).

Nutrient concentrations were not correlated with CT, RGC, $DI_{6.7}$ and WT ($r < 0.25$, $p > 0.65$, $n = 6$), and mean values of SLA (42.2 ± 1.5 m^2/kg for petunia and 13.0 ± 0.6 m^2/kg for geranium) were not significantly different among treatments ($p > 0.05$), hence it is not necessary to deepen the discussion with respect to these parameters. In summary, commercial quality *Petunia* and *Pelargonium* plants can be grown in a substrate containing *S, V,* and *B*, with related or improved appearance over those grown in a peat-based control substrate (*S*). Plants grown with limited ratios of *B* and *V* in the mixtures, when transplanted or exposed to abiotic stress, also showed a similar or occasionally enhanced physiological status to plants grown in a peat-based control substrate. This statement is based on the fact that: the addition of *V* and *B* to the substrate enhanced SDW and flower production; RGC did not vary significantly except for 78:10:12 in *Petunia*, which was 73% higher than the control; and DI_{67} and CT did not show significand differences among substrate treatments for both species.

On the other hand, when vermicompost and biochar partially replace peat-based substrates, there is a Carbon storage potential per pot transplanted into the bedding area in the garden. A 800 ml container may store up to 88.74 gr of CO_{2e} for long periods of time (Alvarez JM *et al.*, 2017).

Conclusions

Plant size and flower production improved when peat-based substrate was substituted by vermicompost and biochar at rates of $B \leq 12$ % and $V \leq 30$ % volume fraction. No similar results have been found to date in container production of ornamental plants. Growers of *Petunia* and *Pelargonium* as well as other container plants may benefit from these findings. The changes in the considered physiological parameters, showed that plants grown in these new substrates will be able to adapt themselves, at least similarly well as the plants grown in peat-based growing media, to the new environment after transplanting to garden soil. These outcomes are pertinent to reduce peat usage in container production of ornamental plants and store carbon (C) for long time-periods in urban areas after bedding plants were transplanted to gardens. These facts are also relevant to lowering inorganic fertilization, as vermicompost can provide the required plant nutrients. As biochar is a highly variable product, depending on the feedstock material and pyrolysis conditions, the present results advocate for its use as a component of growing media, but more extensive research should be carried out to maximize both its environmental and agronomical benefits.

Conclusiones

El tamaño de la planta y la producción de flores mejoraron cuando parte del sustrato a base de turba se sustituyó por vermicompost y biochar en proporciones de volumen de $B \leq 12$ % y $V \leq 30$ %. Hasta la fecha no se han encontrado resultados similares en la producción de plantas ornamentales en contenedor. Los productores de *Petunia* y *Pelargonium*, así como de otras plantas en contenedor, pueden beneficiarse de estos hallazgos. Los cambios en los parámetros fisiológicos considerados mostraron que las plantas cultivadas en estos nuevos sustratos podrán adaptarse al nuevo entorno después del trasplante al suelo de jardín, al menos de manera similar, a las plantas cultivadas en medios de cultivo a base de turba. Estos resultados son pertinentes para reducir el uso de turba en la producción en contenedores de plantas ornamentales y almacenar carbono (C) durante largos periodos de tiempo en áreas urbanas después de que las plantas de arriate se trasplanten a los jardines. Estos datos también son relevantes para disminuir la fertilización inorgánica, ya que vermicompost puede proporcionar los nutrientes necesarios para las plantas. Como el biochar es un producto altamente variable, dependiendo de la materia prima inicial y de las condiciones de la pirolisis, los resultados actuales abogan por su uso como componente de sustratos de cultivo, pero se debe realizar una investigación más extensa para maximizar sus beneficios ambientales y agronómicos.

CHAPTER 3

Vermicompost and biochar substrates can reduce nutrients leachates on containerized ornamental plant production

Resumen

La producción de plantas ornamentales en contenedor se enfrenta a varios desafíos ambientales. Uno de ellos es el de reemplazar los ampliamente utilizados sustratos a base de turba, pero que tienen una cuestionable sostenibilidad, y otro es el de evitar la contaminación del agua por los nutrientes que se lixivian del vivero. Por lo tanto, como se ha verificado que las plantas de petunia y geranio pueden producirse en sustratos basados en turba parcialmente reemplazados por vermicompost (*V*) y biochar (*B*) sin disminuir la calidad comercial, este estudio se ha centrado en analizar el lixiviado de un sustrato estándar basado en turba, tomado como control, utilizado en viveros comerciales para producir estas dos especies ornamentales, y aquellos lixiviados procedentes del mismo sustrato al que se han agregado diferentes proporciones en volumen de *V* (10 % y 20 %) y *B* (4 % y 12 %). Se ha verificado que la cantidad de nitrógeno lixiviado de los sustratos mixtos se redujo en comparación con el control en ambas especies (un 37 % de promedio). El nitrógeno se lixivió principalmente en forma de nitrato (89 % en *Petunia* y 97 % en *Pelargonium*). En *Petunia*, la lixiviación de fósforo también disminuyó (30 %) para el tratamiento con 10 % de *V* y 4 % de *B*, mientras que la lixiviación de potasio en un sustrato que contenía 20 % de *V* y 12 % de *B* aumentó en un 100 %. Nuestros resultados muestran que estos dos materiales orgánicos probados (*V* y *B*) pueden ayudar a reducir el uso de turba y fertilizantes químicos, así como a reducir el riesgo de contaminación por sustancias químicas, principalmente de nitratos.

Abstract

Containerized ornamental plant production is facing several environmental challenges. One of them is to replace the widely used, but with questionable sustainability, peat based substrates and another is to avoid water contamination by chemicals leaching from the nursery. Therefore, as have been verified that petunia and pelargonium plants can be produced in peat-based growing media partially replaced by vermicompost (*V*) and biochar (*B*) without decreasing commercial quality, this study has focused on analyzing the leachate from a standard peat-based substrate as a control, used for producing these two ornamental species, and those from the same substrate to which different proportions in volume of *V* (10 % and 20 %) and *B* (4 % and 12 %) have been added. It has been found that the amount of nitrogen leached from the mixed substrates was reduced compared to the control one in both species (on average 37 %). Nitrogen was leached mainly as nitrate-nitrogen (89 % in *Petunia* and 97 % in *Pelargonium*). In *Petunia* phosphorous leaching was also decreased (30 %) for the treatment with 10 % *V* and 4 % *B*, while potassium leaching in substrate containing 20 % *V* and 12 % *B* increased by 100 %. Our results show that these two organic materials tested (*V* and *B*) can help producers to reduce the use of peat and chemical fertilizers as well as the risk of contamination by chemicals, mainly nitrate.

Introduction

Containerized ornamental plants growers have to face several environmental challenges both to compile legal requirements and the increasing environmental demands of their customers. We can mention one on which the producer will have sooner or later to make decisions about due to the peculiarities of this type of containerized ornamental plants production (Ruter, 1993). Irrigation and fertilization management should be adequate to avoid nutrients leaching to public waters adjacent to the area of the production facilities and their eventual contamination (Cabrera, 1997, Majsztrik *et al*., 2011). Actually, in Europe and the United States there is an increasing pressure to reduce the leachates of horticultural crops for environmental reasons (Guimera *et al*., 1995). Nitrate, ammonium and phosphates are the ions that are considered the most problematic irrigation leachates (Mueller *et al*., 1995) due to their effect in surface waters and impact in public health (Agegnehu *et al*., 2017).

Our hypothesis is that the inclusion of biochar and vermicompost, in a peat based growing media could reduce the leaching of nutrients while maintaining an adequate plant quality. Our main objective in this study is assessing the leaching of nitrogen and other nutrients from peat based blends including biochar and vermicompost in comparison with usual fertilized peat substrates.

Material and methods

Plant material and experimental design

Two ornamental species very much worldwide used were utilized, *Petunia x hybrida* cv. Dreams Neon and *Pelargonium peltatum* cv. Summer Showers. These species were also chosen for their different nutrients needs and rusticity as well as on their salt tolerance, being *Petunia* more tolerant than *Pelargonium* (Mionk & Wiebe, 1961; Do & Scherer, 2013), since *V* and *B* could modify mineral nutrients availability, electrical conductivity and pH (Alvarez JM *et al*., 2017).

Commercial products available in the market were used to make up the growing media, biochar (*B*), vermicompost (*V*) and a peat-based substrate (*S*). The biochar is called Soil Reef Pure 02 (Biochar Solutions Inc., Co, USA) and was produced by high temperature pyrolysis, 600 to 800 ºC, of *Pinus monticola* wood. The vermicompost is named Black Diamond Vermicompost (Black Diamond Vermicompost, Ca, USA) and was produced by pre-composting for two weeks the solid fraction of bovine manure using an aerated composting system, then submitted to a vermicompost process for a period of 70 to 80 days. These two renewable organic materials (*B* and *V*) were used to partially replace a peat-based control substrate (*S*) called Farfard 3B mixture (SunGro Horticulture Distribution Inc., USA). This peat-based substrate is composed by Canadian *Sphagnum* peat moss, pine bark, perlite, vermiculite, dolomitic limestone, and a wetting agent, at 6:4:2:1 Peat:Bark:Perlite:Vermiculite volume ratio. Farfard 3B received a slow release fertilizer (Scotts Osmocote Plus 15-9-12, 5-6 months release at 21˚C, at a dosage of 5.9 g L^{-1}). An overview of the main characteristics of these components, and more details appear in chapter 1 and in Alvarez JM *et al.* (2017).

Three growing media (mixes) were prepared with the following volume fractions (*S*:*V*:*B*): 100:00:00, 86:10:04 and 68:20:12, being, respectively, the control treatment and two treatments containing a slight and a moderate peat-based substrate replacement. The last two treatments were selected based on the previous study (see chapter 1) when 23 different mixes were compared with *S* (i.e. *S* = 100:00:00 treatment), and according to the good plant growth and flowering obtained (Alvarez JM *et al.*, 2017). Then, bulk density (*Db*), water holding capacity (*WHC*), total porosity (*Pt*) and air space (*As*) were determined at the beginning of the experiment following the procedures for determining physical properties of horticultural substrates using the NCSU porometer (Fonteno & Bilderback, 1993). Soluble nutrients, pH and electric conductivity (EC) were determined in aqueous extracts (1:6 volume fraction) taken from fresh mixtures samples in advance of plants cultivation: nitrate and ammonium by spectrophotometry in a flow autoanalyser (AA III, Bran + Luebbe, Norderstedt, Germany) (Ansorena Miner, 1994); potassium, sulfate and phosphate by ICP-OES (Dahlquist & Knoll, 1978); EC and pH by a pH-meter/conductimeter (Acumet® Ap85, USA) (Ansorena Miner, 1994).

Petunia and *Pelargonium* seeds were germinated in 100 plug trays (21.8 cm^3 per cell) and was added two seeds per cell. After germination, just one seedling was kept. Trays were placed in a glasshouse for 40 days at 24 °C and 54 % average temperature and relative humidity, respectively under a climate control system in the greenhouse. Watering was done with an automatic micro sprinkler irrigation system between dawn and dusk. Nozzles were irrigating at 1.8 L h^{-1} during 15 seconds every 20 min, with 2 m diameter and 1 meter overlap. After that, thirty seedlings were randomly obtained, transplanted into 800 cm^3 plastic containers and moved to a glass covered greenhouse (average temperature 20 °C and average humidity 29 % also under a climate control system in the glasshouse) for 68 days until the market size was reached. Standard propagation protocols for these species were followed. The experimental design was a completely randomized design with two replicas. Each replica consisted of 5 plants per species and treatment randomly distributed (5 plants x 3 treatments x 2 species = 30 plants per replica). The two replicas were placed on separate benches (2 replicas x 15 plants = 60 plants). Plants were rotated periodically to minimize variation in microclimatic conditions. Containers were watered manually as needed with distilled water. The water was added to each pot gradually by using a slight volume every time ($\leq$ 10 cm^3) and waiting for a few minutes before adding next volume. As soon as a water droplet appeared at the bottom of the pot no more water was added.

These few water droplets leached from each pot were taken back to the pot. Therefore, water was gradually added trying to avoid leaching and to keep substrate to field capacity.

Plant growth, leaching parameters and data analysis

The parameters evaluated were shoot dry weight (SDW) of plants, and containers leachates volume and nutrient contents. At the end of the growing period and before measuring shoot dry weight (SDW) of plants, containers leachates were collected during five consecutive days after receiving a daily watering of 200 cm^3. In order to collect the leachate, both a plastic mesh and a plastic cuvette were placed under each container. For every sampling date, the substrate was moistened to field capacity, as described before, one day before to collect the samples. Collected volume was measured and a sample was taken for subsequent nutrient analysis of nitrate-nitrogen ($N\text{-}NO_3^-$), nitrite-nitrogen ($N\text{-}NO_2^-$), ammonium-nitrogen ($N\text{-}NH_4^+$), phosphate-phosphorous ($P\text{-}PO_4^{-3}$), total P, sulfate (SO_4^{-2}). The total nitrogen was calculated as the sum of nitrate-, nitrite-, and ammonium-nitrogen. The nutrient contents (mg) collected in the leachates were calculated by multiplying the concentration (mg L^{-1}) by the collected volume (L). Nutrient analysis was performed by means of standard methods using a multiparameter photometer (HI 83200, Hanna Instruments®, Italy).

At the end of the growth period SDW and number of flowers were recorded in *Petunia* and *Pelargonium* plants. In pelargonium number of open inflorescences and inflorescence-buds were also counted. Shoot dry weight was obtained after oven-drying at 55 °C for 72 h. For SDW and inflorescences, one-way analysis of variance (SPSS Statistics 17.0) were carried out to determine statistically significant differences between treatments, being the treatment a fixed effect. While for leachate nutrient concentrations and nutrient contents repeated measured ANOVA were carried out, since nutrient concentration in the leachate on a specific day depends on the concentration obtained in previous days. Significant differences were established at $p = 0.05$. To evaluate the among treatments comparisons, Tukey-HSD or T3-Dunnett tests were used in order to differentiate within homogeneous groups, according to variance homoscedasticity. In addition, correlation and regression analysis were performed in order to establish the underlying relationships between treatments and measured parameters.

Results and discussion

Physical characteristics of the substrates and plant growth

The physical properties at the beginning of the experiment of peat-based substrate (*S*), and the two different mixtures studied are shown in Table 3.1.

Table 3.1: Physical properties, mean (SE), of growth media (treatments) used in the experiment.

	Db	***WHC***	***Pt***	***As***
S:V:B	**kg dm^{-3}**	**%**	**% v/v**	**% v/v**
100:00:00	0.140 (0.03) a	70.1 (0.6) a	80.1 (0.2) a	10.3 (0.9) a
86:10:04	0.143 (0.05) a	71.5 (0.7) a	80.3 (0.6) a	8.7 (1.2) a
68:20:12	0.154 (0.02) b	72.2 (0.6) a	80.7 (0.8) a	8.2 (0.3) a
p	0.02	0.12	0.87	0.30

Db = bulk density; *WHC* = water holding capacity; *Pt* = total porosity; *As* = air space.
S = peat-based substrate, *V* = vermicompost, *B* = Biochar. Control, 100:00:00. Volume fraction (%).
p = significance level. Different letters in numerical columns differ at $p \leq 0.05$ (Tukey-HSD test for *Va*, *Pt* and *As*; T3-Dunnett test for *Db*). Columbus. OSU. 2016

Pt and *As* in all mixtures lied within the suggested optimum ranges, 68 to 88 % and 6 to 13 %, respectively. *WHC* was always slightly above the recommended range 45 to 65 %, while *Db* was also slightly above the recommended range (100 to 140 kg m^{-3}), except for control. All the above met the recommendations made by Bilderback *et al*. (2005) and Yeager (2000).

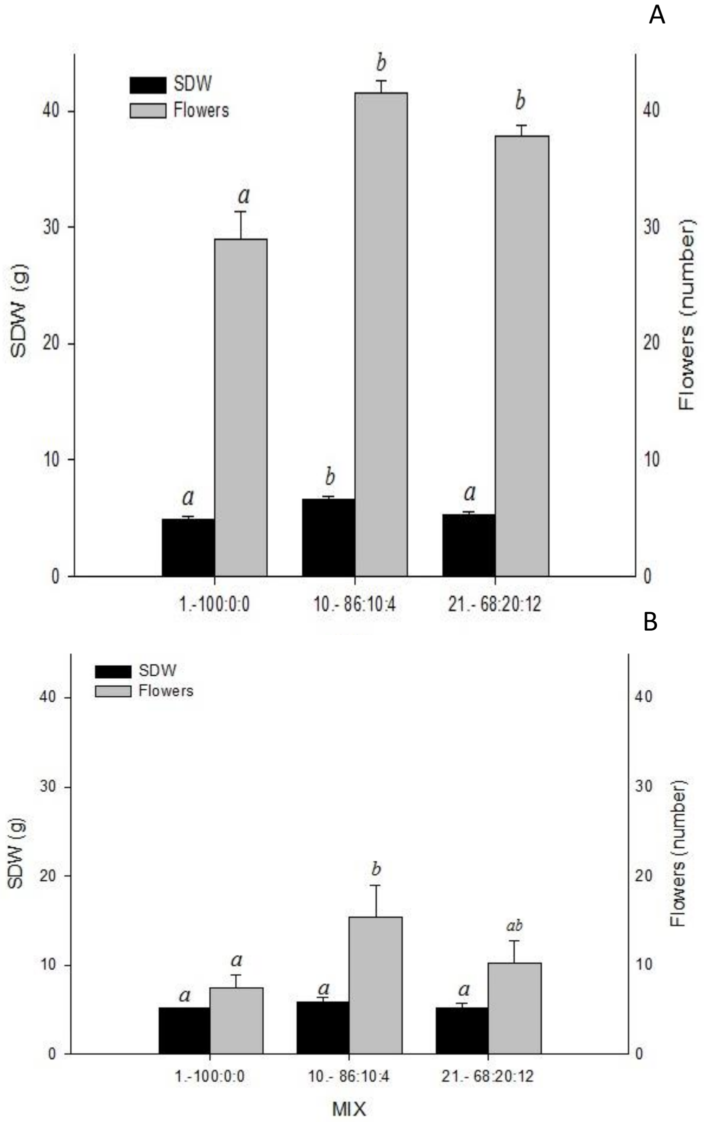


Figure 3.1: Shoot dry weight (SDW, g) and flower production number of petunia (A) and pelargonium (B) grown in mixtures with different proportions of peat-based substrate (*S*), vermicompost (*V*) and biochar (*B*) (*S*:*V*:*B*). Letters show significant differences between substrates studied ($p < 0.05$). (Tukey-HSD test for SDW both species, and for Flowers in *Petunia*; T3-Dunnet test for Flowers in *Pelargonium*). Columbus, OSU, 2016.

Figure **3.**1 shows accumulated plants biomass and number of flowers per plant for the two ornamental species grown in the three different treatments. *Petunia'*s SDW and flowering were significantly higher in mixture 86:10:04 compared with control. Treatment 86:10:04 grown up to 37 % and produced 43 % more flowers than the standard peat based substrate. Mix 68:20:12 produced 30 % more flowers than control. In the case of *Pelargonium*, SDW was similar in all treatments, but flowering in mix 86:10:04 significantly and positively differed from the control, producing up to 108 % more flowers. In regard to physical and physico-chemical characteristics of these three substrates, only bulk density (D_b) was affected by the addition of *V* and *B*, being the heaviest mixture (68:20:12) only a 10 % heavier than the control one. The addition of *V* to peat substrates usually increase D_b (Mupondi *et al.*, 2014; Álvarez JM *et al.*, 2017), but in this study, taking into account the proportions of *V* used, it does not seem to have negatively affected the plant growth and nursery management. In respect of plant growth and flower production, our results clearly showed that *Petunia* and *Pelargonium* growth and flowering status was enhanced with the inclusion of *B* and *V* in peat based substrate in slight or moderate proportions. These results are aligned with other species (Graber *et al.*, 2010; Tian *et al.*, 2012; Mulcahy *et al.*, 2013). For instance, Graber *et al.* (2010) found an increase in pepper canopy dry weight and flowering by the addition of biochar to a coconut fiber:tuff mix; Tian *et al.* (2012) obtained similar results growing *Calathea rotundifolia* plants in 50 % green waste pyrolyzed biochar added to a peat medium, compared to 100 % peat; and an improvement in tomato plant height in growing medium amended with wood pyrolyzed biochar (1 to 5 %, weight fraction).

Leachate properties

On average, *Pelargonium*'s leachate volume per pot and date (50.6 cm^3) was 47 % lower than *Petunia*'s (74.4 cm^3). For both species, neither the effect of treatment ($p \geq 0.107$) nor treatment x date interaction ($p \geq 0.561$) were significant (Figure 3.2). However, the sampling date was significant ($p \leq 0.005$): for *Pelargonium* it ranged from 33.9 cm^3

(day 3) to 68.4 cm^3 (day 1), whereas for *Petunia* it did from 40.5 cm^3 (day 1) to 107.7 cm^3 (day 5), but without following a defined pattern between consecutive days.

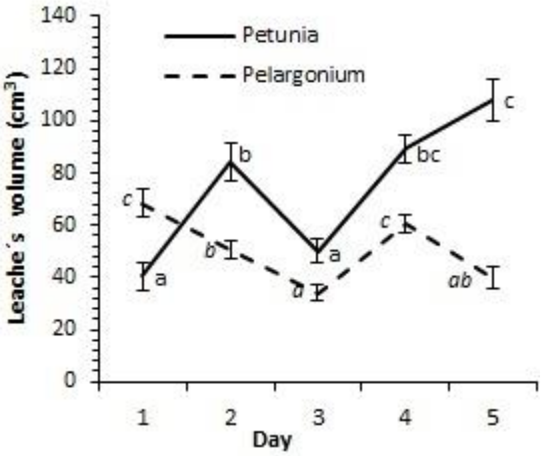


Figure 3.2: Leachate´s volume (cm^3) of petunia and geranium grown in mixtures with different proportions of peat-based substrate (*S*), vermicompost (*V*) and biochar (*B*), (*S*:*V*:*B*). For each species letters show significant differences among sampling dates ($p < 0.05$). Huelva, ETSI, 2017.

For both species, collected leachates did not show significant differences in pH between sampling dates ($p \geq 0.165$) nor for treatment x date interaction ($p \geq 0.405$), but the effect of treatment was significant ($p < 0.001$). The ranking between treatments was 100:00:00 < 86:10:04 < 68:20:12, with values around neutral, slightly lower for *Pelargonium* (6.5 < 7.1 < 7.5, respectively) than for *Petunia* (7.0 < 7.6 < 7.9, respectively). The increase in pH was well correlated to both components added to peat-based substrate. In *Petunia,* pH was significantly related to *B* ($R^2 = 0.72$, $p < 0.01$, n = 30) and to *V* ($R^2 = 0.79$, $p < 0.01$, n = 30). Also in *Pelargonium,* pH was related to *B* ($R^2 = 0.72$, $p < 0.01$, n = 30) and *V* ($R^2 = 0.69$, $p < 0.01$, n = 30).

EC was higher in *Pelargonium* (4.3 ± 0.2 dS m^{-1}) than in *Petunia* (1.9 ± 0.1 dS m^{-1}), with no significant differences between sampling dates ($p \geq 0.155$) nor between treatments for *Pelargonium* ($p = 0.415$). However, the treatment effect was significant for *Petunia* ($p = 0.012$). The mean values for *Petunia* were 1.7, 1.9 and 2.1 dS m^{-1} for 68:20:12, 86:10:04

and 100:00:00, respectively, being significant the differences between the two most extreme treatments.

The treatment x date interaction was not significant ($0.063 < p < 0.873$) in mineral nutrients concentrations and contents in leachate. Leachate´s concentration of sulfate ions (SO_4^{-2}) did not differ significantly between treatments for either species ($p \geq 0.884$), but there was difference between species, resulting in a 27.6 % higher for *Pelargonium* 401 mg L^{-1} than for *Petunia* 314 mg L^{-1}. However, sampling date was significant ($p \leq 0.038$) for sulfate ions, as concentration decreased from the first to the last date: 446 to 343 mg L^{-1} for *Pelargonium* and 363 to 240 mg L^{-1} for *Petunia*. Total sulfur´s amount (S, contained in sulfate ions, i.e. $S\text{-}SO_4^{-2}$) per pot, as the sum of the five days sampled, averaged 35 mg for *Pelargonium* and 38 mg for *Petunia*.

Table 3.2 shows N, P and K leachates concentration values. N concentration in leachates was reduced in the mixed substrates compared to the control one in both species, while K concentration increased. In the case of N, concentration decreased 18 to 22 % in *Petunia,* and 17 to 40 % in *Pelargonium*.

Whereas for K, the increments were 97 % in *Petunia,* but only significant for the 68:20:12 treatment, and 29 to 53 % in *Pelargonium.* In *Petunia* phosphate-P form represented 46 % of the total P, whereas for *Pelargonium* it was 61 %. Regarding N, in *Petunia,* 89 % corresponded to nitrate-N, 10.9 % to ammonium-N and the remaining 0.1 % to nitrite-N. In *Pelargonium*, respective percentages were 97 %, 2.9 % and 0.1 %.

Table 3.2: Concentration, mean (SE), of N, P and K in the leachate collected from each pot for the different treatments and sampling dates . Huelva, ETSI, 2017.

(mg L^{-1})	*Petunia*			*Pelargonium*		
	N	**P**	**K**	**N**	**P**	**K**
Treatment						
100:00:00	52.1 (3.8) b	23.1 (0.7) a	46.5 (4.4) a	247 (14) c	18.2 (1.3) a	208 (22) a
86:10:04	40.8 (4.0) a	21.6 (0.6) a	47.1 (3.6) a	205 (13) b	19.0 (0.9) a	269 (21) ab
68:20:12	42.9 (2.5) ab	24.4 (0.9) a	91.6 (5.6) b	148 (9) a	18.5 (1.2) a	318 (28) b
p	0.031	0.052	0.034	0.016	0.958	0.034
Date						
1st day	55.7 (5.5) c	21.5 (1.1) a	71.9 (9.3) b	246 (28) b	19.7 (1.5) a	356 (32) b
2nd day	53.1 (3.8) bc	22.7 (0.7) a	69.7 (8.0) ab	230 (21) ab	19.3 (1.4) a	290 (21) b
3rd day	48.2 (3.6) bc	23.1 (1.2) a	66.5 (9.8) ab	190 (14) ab	20.1 (1.7) a	229 (27) b
4th day	39.6 (4.0) ab	23.3 (0.6) a	56.9 (6.9) ab	180 (11) a	18.1 (1.1) a	203 (22) ab
5th day	30.8 (4.3) a	24.5 (1.4) a	48.9 (6.9) a	173 (12) a	15.6 (1.4) a	186 (23) a
p	0.006	0.131	0.039	0.013	0.056	0.003

p = significance level at 0.05. Different letters in numerical columns differ at $p \leq 0.05$ (Tukey-HSD test).

Figure 3.3 shows N, P and K total amount leached per pot during the five sampling days. The amount of nitrogen leached from the mixed substrates was reduced compared to the control one in both species (32 to 43 % in *Petunia* and 26 to 47 % in *Pelargonium*). These reductions were greater than the 14 and 32% reduction that could be attributed to the dilution of the control substrate in the mixtures 86:10:04 and 68:20:12 respectively. In *Petunia* phosphorous decreased (30 %) for the 86:10:04 treatment, while potassium in 68:20:12 treatment increased by 100 %. Nutrients leached amount measurement related to the inorganic fertilizer added to the peat-based substrate and how much was a contribution of either *V* or *B* was not performed. In particular, *V* contained a large amount of N, P and S, while *B* of K, P and S. For instance, in the case of N, the peat-based substrate together with the 5.9 g/L of inorganic fertilizer added implied 892 mg/L of soluble N (1857 mg/L of total N) in that substrate, while *V* contained 408 mg/L of soluble N, and 3799 mg/L of total N.

Therefore, *V* contained less soluble N but more total N to be released slowly over time. Anyway, it is clear that there has been an interaction in the nutrient retention capacity between the different components of the substrate mixture, since: i) the amount of added inorganic fertilizer was reduced, regarding control treatment, 14% for 86:10:04 and 32% for 68:20:12; ii) water leached by alternative treatments, regarding control, presented, in general, a lower concentration of N, greater than K and equal to P and S; iii) in terms of total amount of nutrients leached (Fig. **3.**3) percentage reduction of N and P in the two alternative treatments was greater than the reduction of added fertilizer. In any case, as SDW and the number of flowers were not decreased, the overall response of the two mixtures containing *V* and *B* seems to be environmentally more attractive than peat based substrate to which soluble inorganic fertilizer need to be added, at least for nitrates and phosphates.

Taking into account the correlation analysis performed between leachate parameters, pH and EC, it can be highlighted that: a) for both species, the total amount of nutrients in each leached sample (N, P, K, S) were positively correlated between themselves ($0.49 < r < 0.89$, $p < 0.001$, $48 < n < 75$); b) for both species, N content and N concentration were negatively correlated with pH ($-0.67 < r < -0.43$, $p < 0.025$, $n = 30$); for *Pelargonium*, EC was positively correlated with N, K and S concentrations and content ($0.44 < r < 0.79$, $p < 0.023$, $n = 30$).

Regarding leachates, the slight pH increase (an increment of only about 1.0) when *V* and *B* were added to the standard peat based growing media shows the capacity of *B* and *V* to serve as a liming agent when added to a peat-based substrate, in addition to their effects on the physical properties (Northup, 2013).

In reference to nutrient content in leachates, it was observed that less quantity of N, K and S has been leached in petunia compared to pelargonium. This fact also coincides with a remarkable greater production of flowers in the former species. In addition, the lower N and S concentrations in the leachates from *Petunia* (and therefore lower nitrate-N and sulfate-S) may be related to the higher pH compared to *Pelargonium*. Likewise, the higher N, K and S concentrations in the *Pelargonium* leachates compared to *Petunia*, may have influenced the positive relationship between these nutrients and EC in the former species.

The fact is that N concentration (Table 3.2) and N content (Figure 3.3) in leachates significantly decreased for both species as *V* and *B* increased, which could be due to nitrate retained to the biochar-vermicompost ensemble and more slowly released (Altland & Locke, 2013; Kammann & Clough, 2014).

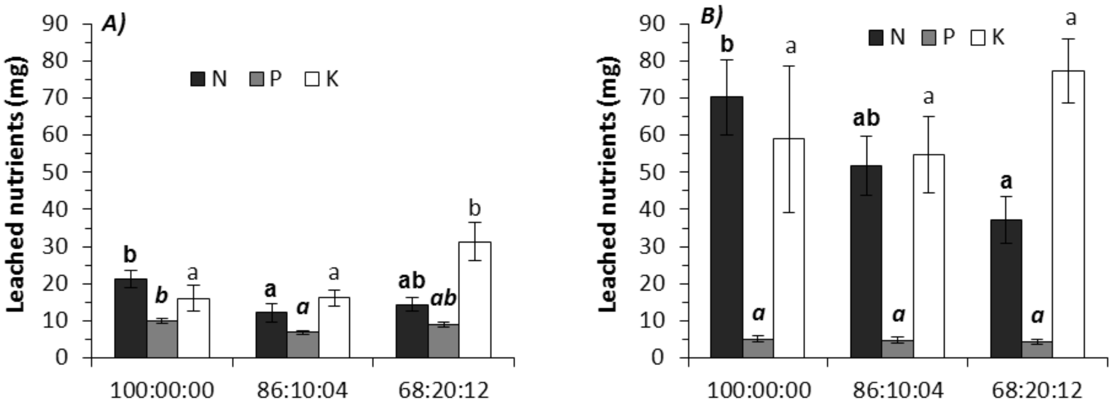


Figure 3.3: Total amount of nutrient leached by containers taken into account the five sample days. Letters show significant differences between substrates studied ($p < 0.05$), Tukey-HSD test. (*A*) *Petunia*, (*B*) *Pelargonium*. Huelva, ETSI, 2017

On the other hand, the increase of potassium concentration in leachates (and content for *Petunia*) as the ratio of biochar applied to the mixtures was also observed in Malińska *et al*. (2016), in which it was noted that biochar could be a significant source of K and should be accounted for in fertility programs (Altland & Locke, 2012). It is not considered necessary to establish a health-based guideline value for potassium in drinking-water. Although potassium may cause some health effects in susceptible individuals, potassium intake from drinking-water is well below the level at which adverse health effects may occur (WHO, 2009). *Petunia*´s leachates, even if higher in volume, had less N and K concentration and content than *Pelargonium*´s probably due a minor nutrients need of last specie to grow and produce flowers (Karras *et al*., 2016). Therefore, the species grown in the pot can also affect the leachate mineral composition.

Conclusions

This study has verified a partial reduction of nitrogen (mainly nitrate) in both species, and slightly P in *Petunia*, leached from the containers as consequence of the biochar-vermicompost inclusion in the selected mixtures additional to the reduction due to the lower ratio of the control substrate in the mixtures. Also, biochar addition could be a significant source of potassium in growing media and may be considered in fertility programs. So, first section of our hypothesis was partially demonstrated.

Obtaining commercial quality plants with similar or even greater growth and flowering than control substrate has served to evidence our second section of our hypothesis that renewable materials can be used for the production of these containerized ornamental plants.

Finally, as biochar produced from high temperature pyrolysis had more recalcitrant character for carbon sequestration and was able to store carbon in soil for longer periods of time (Jindo *et al.*, 2016), so the third section of our hypothesis - climate change mitigation by reducing carbon foot print in this commercial sector - has also been positively addressed.

Conclusiones

Este estudio ha verificado una reducción parcial de los lixiviados procedentes de los contenedores. Esta reducción ha sido de nitrógeno (principalmente nitrato) en ambas especies y levemente de fósforo en *Petunia*. Se ha producido este efecto como consecuencia de la inclusión de biochar-vermicompost en las mezclas seleccionadas así como a la reducción debido a la menor proporción de sustrato de control en las mezclas. Además, la adición de biochar podría ser una fuente importante de potasio en los medios de crecimiento y podría considerarse en los programas de fertilización. De este modo, la primera sección de nuestra hipótesis ha sido parcialmente demostrada.

La obtención de plantas de calidad comercial con un crecimiento y floración similares o incluso mayores que el sustrato de control ha servido para evidenciar la segunda sección de nuestra hipótesis de que los materiales renovables se pueden usar para la producción de estas plantas ornamentales en contenedores.

Finalmente, como el biochar producido a partir de la pirolisis a alta temperatura ha tenido un carácter más recalcitrante para el secuestro de carbono y ha sido capaz de almacenar carbono en el suelo durante períodos de tiempo más largos (Jindo et al., 2016), la tercera sección de nuestra hipótesis relativa a la mitigación del cambio climático gracias a la reducción de la huella de carbono en este sector comercial - también se ha abordado de manera positiva.

CONCLUSIONES GENERALES

El grupo de estudios expuesto anteriormente ha generado una serie de conclusiones que se detallan a continuación.

El estudio de revisión que informa sobre el estado del arte en este tema, concluyó con la necesidad de llevar a cabo ensayos de investigación dirigidos a verificar la viabilidad del uso combinado de vermicompost y biochar para la sustitución parcial de turba en la producción de plantas ornamentales en contenedor.

Los principales resultados del primer experimento fueron que es posible cultivar plantas ornamentales de arriate como la petunia y el geranio en contenedores, con calidad comercial, utilizando diferentes mezclas de biochar / vermicompost añadidos al sustrato con base de turba. Con este cambio en el sustrato sería posible almacenar hasta 88,74 g de CO_{2e} por contenedor de 800 cm^3 durante largos períodos de tiempo, primero en el contenedor donde se ha multiplicado la planta y luego en el suelo después del trasplante de la misma.

En el segundo experimento, las plantas de *Petunia* y de *Pelargonium* cultivadas en las mezclas de sustratos con biochar / vermicompost que mejor rendimiento mostraron en el primer estudio tuvieron, además, una respuesta fisiológica similar o mejor que las plantas cultivadas en el sustrato comercial basado en turba utilizado como control.

Finalmente, en el tercer experimento se confirmaron una reducción en el volumen de lixiviados y también una disminución en la cantidad de los nitratos en los mismos debido a la inclusión de biochar / vermicompost en los sustratos empleados. Por otra parte se verificó que la adición de biochar puede ser una fuente de fertilizante de potasio.

En definitiva, estos resultados obtenidos con diferentes mezclas de biochar y de vermicompost pueden ser de interés para aquellos que desean:

- reducir el consumo de turba para la producción de plantas ornamentales en contenedor.
- reducir la huella de carbono, e incorporar a los poseedores de jardines donde puedan crecer plantas de arriate a la estrategia biótica global de secuestro de carbono en suelo por largos periodos de tiempo para compensar de este modo la emisión de gases de efecto invernadero a la atmosfera y así contribuir a la mitigación del cambio climático.
- reducir los lixiviados de nitratos de este sector comercial productivo.

Además, a modo indicativo, se puede señalar que considerando que cada año se consumen 11 millones de toneladas de turba en la horticultura. Si el 50 % fuera en floricultura y el 20 % en contenedor y si la turba fuera reemplazada por una mezcla de 20 % de vermicompost y 12 % de biochar, habría un posible almacenamiento máximo de carbono en suelo de un millón de toneladas por año.

OVERALL CONCLUSIONS

The group of studies exposed above has generated a number of conclusions that are detailed below.

The review study informing about the state of art in this topic, concluded with the need to undertake research trials aimed at verifying the viability of the combined use of vermicompost and biochar for the partial substitution of peat in the production of ornamental bedding plants in container.

The three trials described above were therefore defined. After finishing the first experiment it has been possible to affirm categorically that it is possible to cultivate bedding ornamental plants such as petunia and geranium in container with good commercial quality using different mixtures of biochar / vermicompost with a substrate based on peat. The calculation made about potential storage in soil, suggests that it would be possible for long periods of time to store first in the plant´s container and then in urban garden´s soil after transplanting, up to 88.74 g of CO_{2e} per 800 cm^3 container.

The second experiment has demonstrated that *Petunia* and *Pelargonium* plants, grown with the best biochar / vermicompost substrate mixtures of the first experiment, showed a similar or better physiological response than the plants grown on a substrate based on a commercial peat that was used as control.

In the third experiment it has been seen that by using these better mixtures, it is possible to reduce both the volume of leachate from the irrigation and the amount of nitrates contained therein, by including biochar / vermicompost in the mixture with the control substrate. It was also verified that the incorporation of biochar to the substrate can suppose an extra source of potassium fertilization that can be considered when planning the fertilization of the crop.

These results obtained with different mixtures of biochar and vermicompost may be of interest to those producers of bedding ornamental plants in container who wish to:

- reduce the consumption of peat for the production of ornamental plants in containers.
- reduce the carbon footprint , and incorporate the owners of gardens where bedding plants can grow to the global biotic strategy of carbon sequestration in soil for

long periods of time to compensating in this way the emission of greenhouse gases into the atmosphere and thus contribute to the mitigation of climate change.

• reduce nitrate´s leachate of in this productive sector.

In this context it has to be indicatively noted, that if we consider that around 11 million metric tons of peat in horticulture are consumed every year in the world. If it is also considered that 50 % of this amount was used in floriculture and 20 % in container production, then it would be possible to store carbon in urban gardening soil for long periods of time for a maximum value of one million metric tons per year, just by partially replacing the peat of the usual substrate with a mixture of 20 % vermicompost and 12 % biochar.

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ANNEXES

ANNEX 1

Publications derived from the Doctoral Thesis

This doctoral thesis, presented under the title "*Biochar and vermicompost use as peat based growing media partial replacement to produce containerized ornamentals* ", has contributed to the publication of 4 articles in international indexed journals, all with peer-review system, and impact index, three of them indexed in ISI-JCR.

A full copy of these publications is attached in "Annex 5, Supplementary Material". These publications are: ¨A biotic strategy to sequester carbon in the ornamental containerized bedding plant production. A review¨ (published on 10/23/18), ¨Vermicompost and Biochar as growing media replacement for ornamental plant production¨ (published on 06/05/18), ¨ Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture¨ (published on 06/30/18).

Finally, the article "Vermicompost and biochar substrates can reduce nutrient leachates in containerized ornamental plants production" has been accepted on 11/21/18 by the International Journal Horticulture Brasileira and it is under their edition process (Annex 4).

The impact of the journals in which the aforementioned works have been published is detailed in the following two pages.

Report with impact factor of submitted publications

Journals ´s metrics

1.-Spanish Journal of Agriculture Research

Alvarez, J. M.; Pasian, C.; Lal, R.; Lopez-Nuñez, R.; Fernández, M. (2018). A biotic strategy to sequester carbon in the ornamental containerized bedding plant production: A review. Spanish Journal of Agricultural Research, Volume 16, Issue 3, e03R01. https://doi.org/10.5424/sjar/2018163-12871

1.1.- Impact factor (JCR of Clarivate Analytics) : 0.811 29

Category: Agriculture, multidiscipllinary, position 29 of 57, **Q3**

1.2.- SCImago:

SCImago Journal Rank (SJR): 0.037

CiteScore: 1.05

h 5 Index : 18, h 5 median: 22

5-Year Impact Factor: 0.962

Source Normalized Impact per Paper (SNIP): 0.677

2.- Journal of Applied Horticulture

Alvarez J.M., Pasian C., Lal R., Lopez R., Fernandez M.. (2017). Vermicompost and Biochar as growing media replacement for ornamental plant production. J. Appl. Hortic.19(3), 205-214.doi:10.17605/OSF.IO/PZBFS

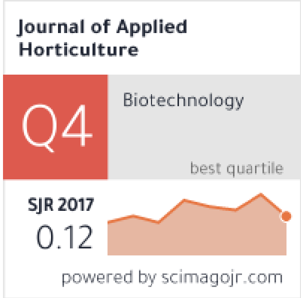
2.1.- Scimago:

SCImago Journal Rank (SJR): 0.124

CiteScore: 0.198

h 5 Index : 6, h 5 median: 7

5-Year Impact Factor: 6



Currently Journal of Applied Horticulture is included in the following Abstracting Services : Index Copernicus, Scopus, Elsevier Bibliographic Databases, EBSCO, Horticultural Abstracts, Chemical Abstracts, Food Science and Technology Abstracts, Agroforestry Abstracts, Agricultural Engineering Abstracts, Crop Physiology Abstracts, Forestry Abstracts, Irrigation and Drainage Abstracts, Indian Science Abstracts, Ornamental Horticulture, Plant Breeding Abstracts, Plant Genetic Resources Abstracts, Postharvest News and Information, Review of Plant Pathology, Seed Abstracts, Soils and Fertilizers, Review of Aromatic and Medicinal Plants, World Agricultural Economics and Rural Sociology Abstracts, Rural Development Abstracts, Review of Agricultural Entomology and Review of Medical and Veterinary Entomology. Indian Science Abstracts.

3.-Urban Forestry and Urban Greening

Alvarez J.M., Pasian C., Lal R., Lopez R., Fernandez M. (2018). Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture. Urban For Urban Green. 34 175-180 https://doi.org/10.1016/j.ufug.2018.06.021

3.1.- Impact factor (JCR of Clarivate Analytics): 2.782

Category: Forestry, position 5 of 66, **Q1**

3.2.- SCImago:

SCImago Journal Rank (SJR): 1.037

CiteScore: 3.22

h 5 Index : 38, h 5 median: 48

5-Year Impact Factor: 3.521

Source Normalized Impact per Paper (SNIP): 1.27

4. Horticultura Brasilera

Alvarez J.M., Pasian C., Lal R., Lopez R., Fernandez M. Nutrients leachates when biochar and vermicompost are used as growing media replacement. (In press). HORTIC BRAS

4.1.- Impact factor (JCR of Clarivate Analytics): 0.677

Category: Horticulture, position 19 of 37, **Q3**

4.2.- SCImago:

SCImago Journal Rank (SJR): 0.605

CiteScore: 0.916

h 5 Index : 10, h 5 median: 13

5-Year Impact Factor: 0.53

ANNEX 2

Journals´s permission to include publications full text in Doctoral Thesis document

A biotic strategy to sequester carbon in the ornamental containerized bedding plant production: A review.

Alvarez, J. M.; Pasian, C.; Lal, R.; Lopez-Nuñez, R.; Fernández, M. (2018). A biotic strategy to sequester carbon in the ornamental containerized bedding plant production: A review. Spanish Journal of Agricultural Research, Volume 16, Issue 3, e03R01. https://doi.org/10.5424/sjar/2018163-12871

Permiso para incluir articulo en tesis doctoral

Jose Maria Alvarez de la Puente sáb., 20 oct. 19:21 (hace 2 días)

Estimada Carmen Recientemente ha sido aceptado para su publicacion en SJAR el manuscrito : A biotic strategy to sequester carbon in the ornamental containerized

Carmen 3:11 (hace 3 horas)

para mi

Estimado Jose María: no necesita nuestro permiso, somos una revista "Open Access" (Articles are distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License), solo debe indicar que el artículo está publicado en nuestra revista, con la cita correspondiente.

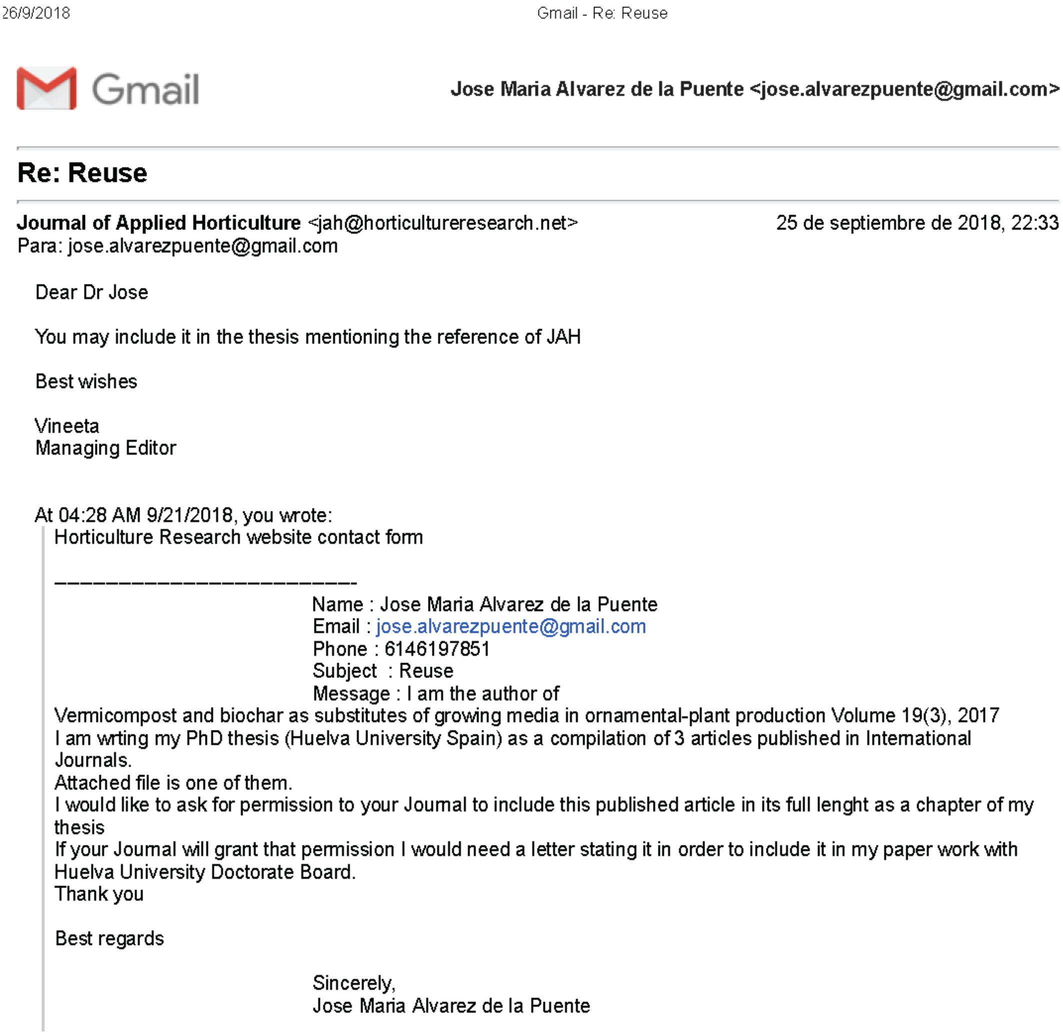
Mucha suerte con la tesis. Saludos,

Dra. Carmen de Blas
Editora
Spanish Journal of Agricultural Research
****

28040-Madrid (Spain)

Vermicompost and Biochar as growing media replacement for ornamental plant production

Alvarez J.M., Pasian C., Lal R., Lopez R., Fernandez M.. (2017). Vermicompost and Biochar as growing media replacement for ornamental plant production. J. Appl. Hortic.19(3), 205-214.doi:10.17605/OSF.IO/PZBFS



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Best wishes

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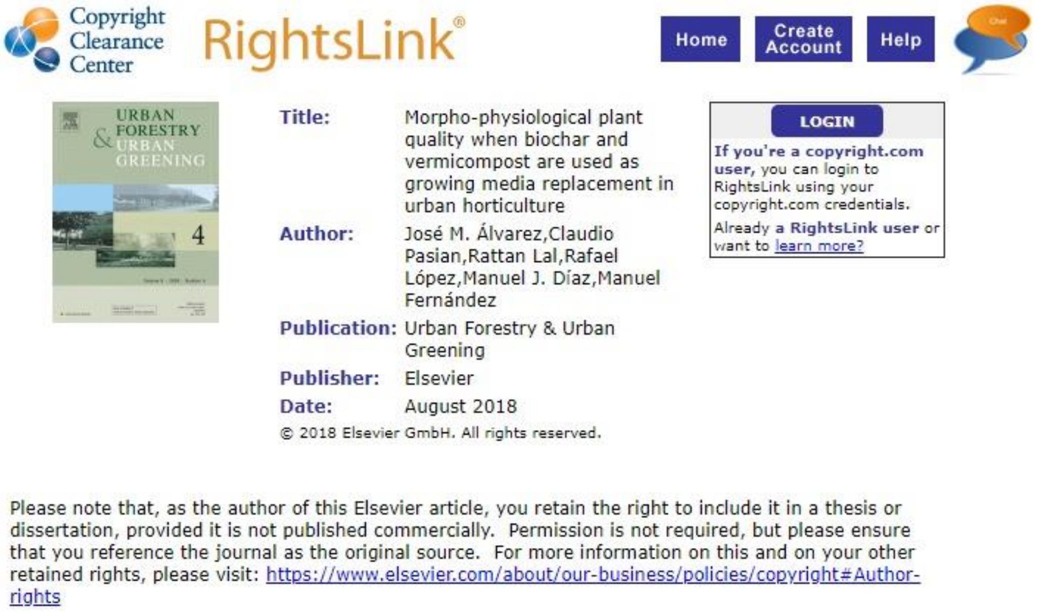
> Thank you
>
> Best regards
>
> Sincerely,
> Jose Maria Alvarez de la Puente

Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture.

Alvarez J.M., Pasian C., Lal R., Lopez R., Fernandez M.. (2018). Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture. Urban For Urban Green. 34 175-180 https://doi.org/10.1016/j.ufug.2018.06.021



Title: Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture

Author: José M. Álvarez,Claudio Pasian,Rattan Lal,Rafael López,Manuel J. Díaz,Manuel Fernández

Publication: Urban Forestry & Urban Greening

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ANNEX 3

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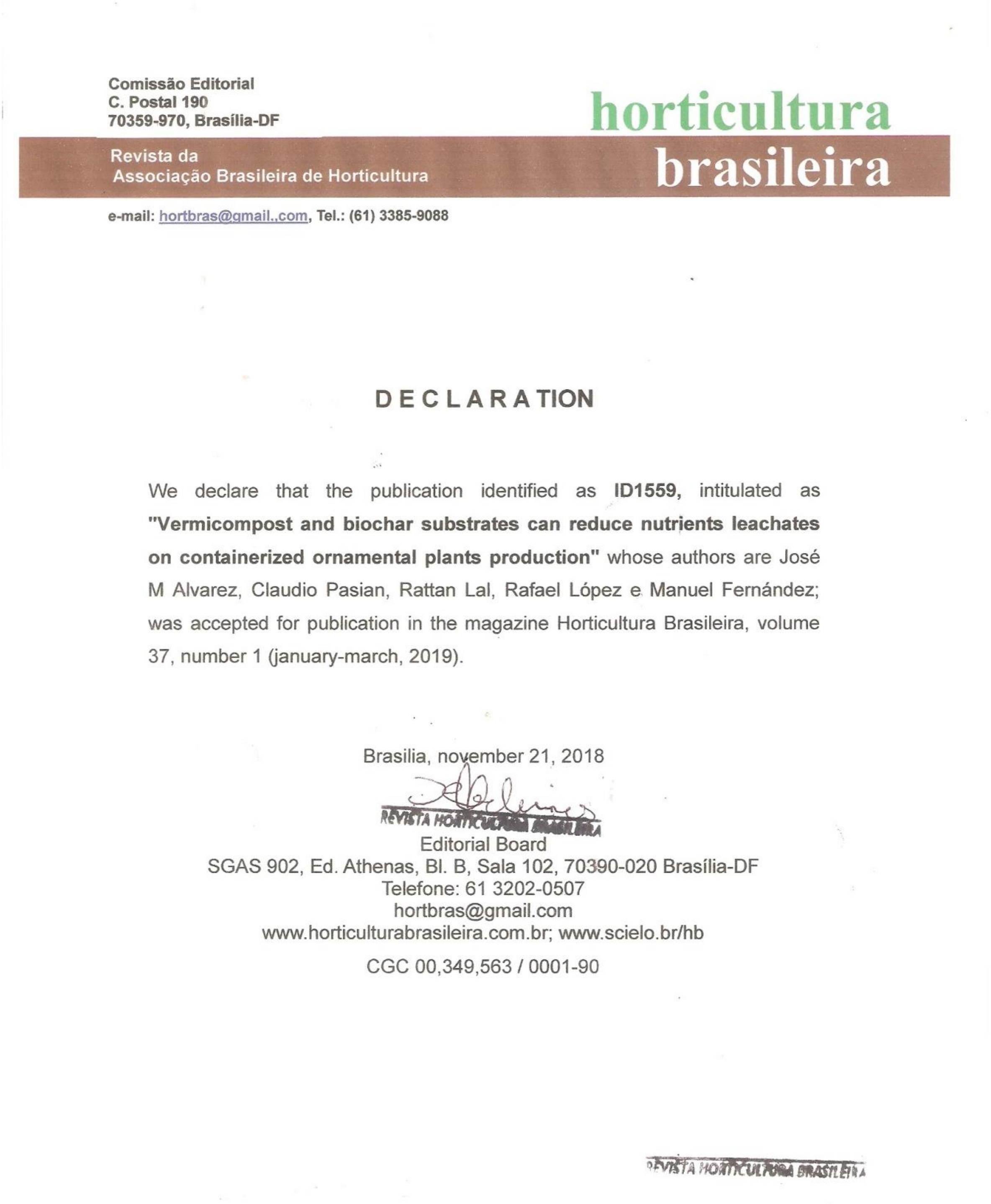

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Revista da
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DECLARATION

We declare that the publication identified as **ID1559,** intitulated as **"Vermicompost and biochar substrates can reduce nutrients leachates on containerized ornamental plants production"** whose authors are José M Alvarez, Claudio Pasian, Rattan Lal, Rafael López e Manuel Fernández; was accepted for publication in the magazine Horticultura Brasileira, volume 37, number 1 (january-march, 2019).

Brasilia, november 21, 2018

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ANNEX 4

Photographic annex

1st Experiment

Pelargonium seedlings in tray 200 plugs

Seedlings after transplanting to 800 ml container place on greenhouse benches

Author collecting data at OSU greenhouse

Petunia growth and flowering results. Left peat based substrate. Right mix 68: 20: 12

2nd Experiment

Petunia seedlings in 200 plugs tray

Petunia containers

Petunia's leaf set up for FIEL test

Petunia seedlings ready for transplant to 800 ml container

Pelargonium containers starting RGC test

Petunia and *Pelargonium* containers ending RGC test

82 10 08 100 00 00 68 20 12

Petunia growth and flowering results. Center peat based substrate.

3rd Experiment

Petunia (left) and *Pelargonium* (right) during leachates collection

Pelargonium during SDW measurements

pH and EC nutrients leachates measurements (Huelva University)

ANNEX 5

SUPPLEMENTARY MATERIAL

COPY OF 3 PUBLISHED ARTICLES

ANNEX 5

Dos de los artículos publicados en el apartado “Annex 5” han sido retirados de la tesis debido a restricciones relativas a derechos de autor. En sustitución de los artículos ofrecemos la siguiente información: referencia bibliográfica, enlace a la revista y resumen.

Álvarez de la Puente, J.M., Pasian, C., Lal, R., López Núñez, R., Fernández Martínez, M.: “Vermicompost and Biochar as growing media replacement for ornamental plant production”. The Journal of Applied Horticulture. Vol. 19 (3), págs. 205-214. http://dx.doi.org/10.17605/OSF.IO/PZBFS

Enlace al texto complete del artículo: http://dx.doi.org/10.17605/OSF.IO/PZBFS

RESUMEN:

Vermicompost is a product derived from the accelerated biological degradation of organic wastes by earthworms and microorganisms. Biochar is a by-product of the C-negative pyrolysis technology for bio-energy production from organic materials. Containerized plant production in floriculture primarily utilizes substrates such as peat moss. Environmental concerns about draining peat bogs have enhanced interests in research on complementary products that can be added to peat. A comparative greenhouse study was conducted to assess the suitability of biochar (B) and vermicompost (V) as partial substitutes for peat-based growing media for ornamental plant production. Different blends of B at a volume fraction of 0, 4, 8, 12 % and V at 0, 10, 20, 30, 40, 50 % were compared to a baseline peat substrate (S) as control in the cultivation of geranium (Pelargonium peltatum) and petunia (Petunia hybrida). Substrates were characterized for physical and chemical properties, plant growth, and flower production. Mixtures with low-medium V levels (10-30%) and high B level (8-12 %) in Petunia and Pelargonium induced more growth and flower production than that of the control. The results obtained with different B and V associations are of interest to those who want to reduce peat consumption for the production of ornamental plants in containers and to reduce carbon footprint of this commercially productive sector.

Álvarez de la Puente, J.M., Pasian, C., Lal, R., López Núñez, R., Fernández Martínez, M.: "Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture". Urban Forestry and Urban Greening. Vol. 34, págs. 175-180, (2018). https://doi.org/10.1016/j.ufug.2018.06.021

Enlace al texto complete del artículo: https://doi.org/10.1016/j.ufug.2018.06.021

RESUMEN:

Peat moss is the most used soilless substrate in the production of container plants in floriculture. Nevertheless, the drainage of peat bogs due to the peat extraction has increased the necessity of seeking products that could replace the peat that is used in plant production. Therefore, a comparative study was conducted to evaluate the effect of a biochar *(B)* - vermicompost *(V)* mixture, as a partial substitute for peat-based substrates, on the morpho-physiological characteristics of ornamental plants. Different blends containing *B* and *V* were compared to a baseline peat-based substrate (*S*) as control in the cultivation of two ornamental bedding plant species that are widely used in urban areas: geranium (*Pelargonium peltatum*) and petunia (*Petunia hybrida*). Plant growth and physiological parameters were assessed. Results showed that it is possible to grow container plants of these two species with commercial quality, using a peat-based substrate mixed with biochar and/or vermicompost (up to 30% *V* and 12% *B*). Plants in these substrates showed a similar or enhanced physiological response to those grown in the control using commercial peat-based substrate.

A biotic strategy to sequester carbon in the ornamental containerized bedding plant production: A review.

Alvarez, J. M.; Pasian, C.; Lal, R.; Lopez-Nuñez, R.; Fernández, M. (2018). A biotic strategy to sequester carbon in the ornamental containerized bedding plant production: A review. Spanish Journal of Agricultural Research, Volume 16, Issue 3, e03R01. https://doi.org/10.5424/sjar/2018163-12871

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Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, O.A, M.P. (INIA)

REVIEW ARTICLE

OPEN ACCESS

A biotic strategy to sequester carbon in the ornamental containerized bedding plant production: A review

Jose M. Alvarez[1,2,3], Claudio Pasian[2], Rattan Lal[3], Rafael Lopez-Nuñez[4] and Manuel Fernández[1]

[1]Universidad de Huelva, Escuela Técnica Superior de Ingeniería, 21071 Huelva, Spain. [2]Ohio State University, Dept. of Horticulture and Crop Sciences, 249B Howlet Hall, Columbus, OH 43210, USA. [3]Ohio State University, C-MASC, 422B Kottman Hall, Columbus, OH 43210, USA. [4]IRNAS-CSIC, Avenida Reina Mercedes, 10, 41012, Sevilla, Spain.

Abstract

Identifying options of climate change mitigation is of global interest to researchers. Whereas wide range of techniques of reducing greenhouse gas (GHG) emissions and carbon sequestration have been studied in row crops and forest systems, little research has been done on the ornamental horticulture. The ornamental industrial sector has indeed some negative impacts on the global environment, but also presents opportunities to reduce GHG emissions and increase C sequestration. Thus the objective of this study was to synthesize the potential contributions of some substrates used in the horticultural sector to carbon sequestration. The specific focus of the review is on the possible use of compost, vermicompost and biochar as soilless substrate substitutes for containerized ornamental plants production. Around 11 million kilograms of sphagnum peat moss are used annually in the world for horticultural production. Therefore, the potential of using compost, vermicompost and biochar as growing media is assessed on the basis of data from greenhouse studies. Peat-based substrate can be substituted up to 30% to 35% by compost or vermicompost and up to 20% to 25% by biochar. Some examples from field studies are included to conduct the life cycle assessment of using these growth media. An estimate of C storage on the long-term basis in soil indicates up to 3 million tons of CO_2 equivalent as the maximum C potential storage per year in the global productive sector if the peat-based growing media are substituted by compost/vermicompost and biochar at the ratios mentioned above. Finally, synergies between compost vermicompost and biochar are discussed when these materials are combined as growing media additives and research gaps in this area of activity have been identified for further research.

Additional keywords: biochar; compost; substrate additive; peat replacement; carbon storage; ornamental containerized plants.

Abbreviations used: CEC (cation exchange capacity); CO_2e (carbon dioxide equivalent); GHG (greenhouse gas); LCA (life cycle assessment); RMP (recommended management practices); SDW (shoot dry weight); SOC (soil organic carbon).

Authors' contributions: The five co-authors participated in all stages of the work, including the conception and design of the research, the revision of the intellectual content and the drafting of the paper.

Citation: Alvarez, J. M.; Pasian, C.; Lal, R.; Lopez-Nuñez, R.; Fernández, M. (2018). A biotic strategy to sequester carbon in the ornamental containerized bedding plant production: A review. Spanish Journal of Agricultural Research, Volume 16, Issue 3, e03R01. https://doi.org/10.5424/sjar/2018163-12871

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Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to José M. Alvarez: josemaria.alvarez254@alu.uhu.es

Introduction

Climate change and CO_2 sequestration

There is a concern in the scientific field about climate change and its present and future impacts on human wellbeing. An increase in the atmospheric concentration of CO_2 may increase the Earth's mean temperature and change the precipitation patterns (IPCC, 2014). Thus, there is a growing interest in identifying strategies of decreasing the amount of atmospheric CO_2 by reducing anthropogenic emissions (Lal, 2009). In the meanwhile, carbon (C) sequestration capacity of natural sinks (*i.e.*, oceans, forests, peat bogs) is also decreasing because of human activities (Raviv, 2013). The process of transfer and secure storage of atmospheric CO_2 into other long-lived C pools that would otherwise be emitted or remain in the atmosphere is called 'carbon sequestration' (Lal, 2008). Therefore, in this context, C sequestration may be a natural or an anthropogenically driven process. The objective of an anthropogenically driven C sequestration process is to balance the global C budget such that future economic growth is based on a 'C-neutral' strategy of no net gain in atmospheric C pool. Such a strategy would necessitate sequestering almost all anthropogenically generated CO_2 through safe, environmentally acceptable and stable techniques with low risks of leakage (Lal, 2008).

Strategies to C sequestration

There are three main strategies of reducing CO_2 emissions to mitigate climate change: (i) reducing global energy use; (ii) developing low or no-C fuel sources; and (iii) sequestering CO_2 from point sources or atmosphere using natural and engineering techniques (Schrag, 2007). Regarding the last option, engineering techniques of CO_2 injection in deep ocean, geological strata, old coal mines and oil wells, and saline aquifers along with mineral carbonation of CO_2 constitute abiotic techniques. These techniques are expensive and prone to leakage. In comparison, biotic techniques are based on natural and cost-effective processes but have finite sink capacity (Lal, 2008).

Thus far, agriculture has been a major source of gaseous emission. Adoption of agricultural best management practices (*i.e.*, conservation agriculture, integrated nutrient management, precision agriculture, cover cropping, agro-forestry, micro-irrigation) can enhance resilience of soils and ecosystems against perturbations and also mitigate climate change. In this context, there are numerous land use and management practices, which must be discouraged. Notable among these are tropical deforestation, drainage of wetlands, cultivation of marginal/poor soils, intensive tillage, removal of crop residues, flood irrigation and biomass burning. Crop residues and animal dung must be used as soil amendments rather than as sources of household energy (Lal, 2013). Carbon sequestration in agricultural soils enhances sustainability of the land use systems. Increasing soil organic carbon (SOC) concentration in the root zone is beneficial in any situation to generate or maintain healthy soils (Lal, 2004a; Pardo *et al.*, 2017) and it also restores environmental quality and associated ecosystem services over the long time horizon (Lehmann, 2009). Carbon sequestration in ecosystems is measured by infrared gas analyzer to measure CO_2 eddy flux (Goulden *et al.*, 1996). In soils, C sequestration is estimated by difference in biomass and soil carbon content over time (Lal, 2004a).

In this regard the “4 per thousand” proposal at the 21st Conference of the Parties to the United Nations (UN) Framework Convention on Climate Change (COP21) in Paris on 2015, has called for a voluntary action plan to enhance SOC content of world soils to a 40 cm depth at the rate of 0.4% per year. The strategy is to promote SOC sequestration through adoption of the above mentioned recommended management practices (RMPs) of C farming (Lal, 2016). Thus, it is important to identify the specific plant cultures with a high capacity of C sequestration; however, the rate of SOC sequestration with adoption of RMPs may depend on soil texture and structure, rainfall, temperature, farming system, and soil management (Lal, 2004b).

Substrates in ornamental horticulture

Much of the research towards reducing GHG emissions and C sequestration has been conducted in row crop and forest systems. In comparison, a limited research has been conducted on the specialty crop industry such as ornamental horticulture. The latter is an industry that impacts rural, suburban, and urban landscapes. Although this industry may have some negative impacts on the global environment (Nicese & Lazzerini, 2013), it also has opportunities to reduce GHG emissions and increase C sequestration (Marble *et al.*, 2011). The horticultural industry was responsible for emitting 8.0 million tons of CO_2 in 1996. This was 12% more than in 1989/90 (RSFGV, 1999), and has been growing since then. The ornamental horticulture global production reached a value of $37.1 billion in 2014. European Union (34.3%), China (15.9%) and USA (13.9%) contributed 64% of the economy (AIPH, 2017). In USA, five states (California, Florida, Michigan, North Carolina, and Ohio) accounted for 69% of that value. Principal plant´s categories are annual bedding/garden plants 33.2%, potted flowering plants 20.9%, indoor/patio use 18.4%, herbaceous perennial plants 14.8%, propagative floriculture materials 0.9%, cut flower 9.7% and cut cultivated greens 2.1%.The wholesale value for annual bedding and garden plants totalled $1.29 billion in 2015. This value represents 69% of the total bedding and garden category. *Petunia* sp., *Geranium* sp., *Viola* sp., *Impatiens* sp. and *Begonia* sp. cultivars were the top five bedding plant crops grown in flats. These cultivars are usually grown in greenhouses. Initially, seeds/cuttings are cultivated in trays. Young seedlings are transplanted into containers/hanging baskets and grown to maturity (USDA-NASS, 2016).

Containerized plant production in horticulture primarily utilizes soilless substrates. In general, these substrates are primarily composed of organic materials such as peat moss and inorganic materials such as vermiculite and perlite (Bilderback *et al.*, 2013). However, to date, little is known concerning the C sequestration potential of the horticulture industry as a whole; which is also critical to assessing its potential contribution to mitigating the climate change (Prior *et al.*, 2011).

It is in this context that the review below is an attempt to synthesize the potential contributions of some substrates used in the horticultural sector to carbon sequestration. The specific focus of the review is on the possible use of compost, vermicompost and biochar as soilless substrate substitutes for containerized ornamental plants production.

Peat environmental concerns and peat substitutes

Nursery and greenhouse activities worldwide have been challenged to optimize their water and nutrients management (Majsztrik *et al.*, 2011). *Sphagnum* peat moss is the main substrate used in horticulture because of its homogeneous and ideal physical characteristics and high nutrient exchange capacity. As much as 10 to 11 Tg of this material may be used annually in the world for horticultural production (http://minerals.usgs.gov/minerals/pubs/commodity/peat/mcs-2015-peat.pdf). Globally, the total volume of materials used in growing media is difficult to estimate because recent data are not available for many areas of the world, including the Americas (both South and North), Australia, as well as Southeast Asia, where the process of growing out of soil has expanded in recent years but mainly into hydroponic systems in China, Japan, Thailand, and Malaysia (Carlile *et al.*, 2015).

Schmilewski (2017) reported that 34.6 Mm^3 of growing media were manufactured on 2013 in Europe, of which 93.8% was organic materials. Peat was the predominant bulky ingredient (75.1%), followed by organic constituents other than peat and compost (10.8%) and then compost (7.9%). An increase of 100% in green compost utilized as growing media in EU occurred since 2005 (Schmilewski, 2009). Traditional peat extracting countries have a strong focus on peat but there is an ever increasing interest and trend to replace peat by using other organic materials including composts. Countries without indigenous peat resources, *i.e.* the Netherlands, Italy and Belgium, also strongly depend on peat as the main growing media constituent. The principal objective of using mineral materials in growing media is to fine-tune their physical properties, and not to replace peat. In countries like Germany, Austria and Italy with emphasis on recycling bio-waste as part of their circular economies, the use of composts in growing media has increased (~ 6% between 2005 and 2013) (Schmilewski, 2009, 2017) and is likely to develop in other EU member states as targeted by the Circular Economy Strategy of the EU (EC, 2015).

In addition, environmental concerns questioning the peat use in horticulture are growing due to the number of environmental services provided by peatlands (Ostos *et al.*, 2008). They include their habitat value, carbon sink function, regulation of the local water regime and quality and flood protection (Alexander *et al.*, 2008). In fact, peat is no longer considered a renewable resource because it requires thousands of years (Hugron *et al.*, 2013) to be able to generate. Although peatlands represent an important component of the global carbon cycle, storing 23 g m^{-2} y^{-1} of C (Waddington *et al.*, 2002), that today means more than 600 Pg C (Harenda *et al.*, 2018), there are serious doubts about how current peatland will evolve under the climate change situation since these systems require very specific levels of moisture, temperature and insolation (Bragazza *et al.*, 2016).

In any case, there is a consensus about the need to find alternatives to peat as growing media for horticulture in order to reduce the current exploitation and degradation of peatlands when they are in phase of extraction (Waddington *et al.*, 2002). This point of view comes not only from the horticulture industry but also because the influence of macroeconomic issues based on the movements of consumers and decision-makers. Therefore, the challenge lies in identifying and using renewable materials with low costs of production and transportation (Gruda, 2011) and those having adequate physical-chemical characteristics. For instance in UK, environmental groups, government, and horticulture companies have organized themselves to recognize the environmental consequences of peat use in horticulture. In fact the industry is looking increasingly towards renewable raw materials such as green compost or processed timber by-products (Michel, 2010; Caron & Rochefort, 2013).

Composts appear to be a sound alternative to peat within growing media, in volumetric ratio anywhere between 30 to 50% (even up to 100% in specific cases), depending on their origin, composition, maturity and end use (Masaguer & López-Cuadrado, 2006; Raviv, 2013). Coco fibres may partly fulfil this role (Abad *et al.*, 2002). However, since the overall peat demand is growing on the market and the volume needed for peat replacement as a component of substrates greatly exceeds the availability of coco resources, replacement by coco will remain to be low. Moreover, it is expected that the price of coco is going to rapidly increase relative to other biomass in such situations (Caron & Rochefort, 2013). Therefore, the principle focus of this study has been on compost, vermicompost, and biochar, which are some of the industrial peat-based growing media substitutes (Carlile *et al.*, 2015).

Compost and vermicompost

Numerous studies have been undertaken to establish the potential substitution of peat with commercial compost and vermicompost, enhancing plant´s rooting and growth while also reducing the negative side effects (Garcia-Gomez *et al.*, 2002; Sardoei, 2014).

The UK was a pioneer in the research of compost as a substitute for peat (Prasad & Maher, 2001) due to the government decision to establish a deadline for the use of peat in horticulture, thus promoting research in this field (Sohi *et al.*, 2013). Compost from garden pruning and maintenance (green compost) was successful in that research and has since been widely used. Also compost of urban organic waste, bio-solids of sewage treatment plants together with green compost have been effectively tested as growing media in the industrial production of horticultural, forestry and ornamental seedlings (López *et al.*, 2005).

As composting technique has been expanding, each region/country has been testing the composting of its organic waste of silvo-agro industrial origin that has had more at hand. For instance, in Spain, the Lourizan Forestry Research Centre worked on composting of pine bark from sawmills (Miranda & Fernandez, 1992) to be used as growing media for forestry seedling. Later this bark-derived compost was used for the production of ornamental woody plants in container. In regions and countries where containerized ornamental production was important, this initiative was emulated by using organic materials from agro-industries. Such as in Valencia region (Spain) where an inventory of organic agro industrial by-products was carried out with the same goal of manufacturing substrates by composting aiming to utilize them in ornamental container production (Abad *et al.*, 2001). Some of these raw materials were included cork powder (Carmona *et al.*, 2003), two-phase olive oil mill waste ("alperujo") (Fernández-Hernández *et al.*, 2013), organic fraction of the guacamole industry (González-Fernández *et al.*, 2015), organic wastes of greenhouse horticultural production (Mendoza-Hernández *et al.*, 2014), citrus pulp (Gelsomino *et al.*, 2010), grape marc (Trillas *et al.*, 2006), brewery sludge (Sánchez-Monedero *et al.*, 2004), etc.

In vermicompost, researchers used different manures for their transformation by means of lombriculture techniques to identify products that could be used in horticulture. So, mainly pig manure (Atiyeh *et al.*, 2000; Arancon *et al.*, 2005; Bachman & Metzger, 2008; Lazcano *et al.*, 2009) and cattle manure (Tringovska & Dintcheva, 2012; Sultana *et al.*, 2015) were used and also sometimes green and vegetable crop wastes (Fornes *et al.*, 2012; Belda *et al.*, 2013; Morales-Corts *et al.*, 2014).

Peat based substrates were substituted at a 30-35% average ratio by compost and vermicompost in the experiences mentioned in Table 1. Both compost and vermicompost trials showed a beneficial effect related to substrate physical properties and different morphological parameters of the tested ornamental plants grown with these new materials. So, better growth (Do & Scherer, 2013; Mendoza-Hernández *et al.*, 2014; Sultana *et al.*, 2015) increases in shoot dry weight (SDW) (López *et al.*, 2003; Belda *et al.*, 2013; De Lucia *et al.*, 2013) and root collar diameter (RCD) (Álvarez *et al.*, 2001), better container capacity (CC) and water holding capacity (WHC) (Tyler *et al.*, 1993) were recorded in different experiments where the peat-based substrate was partially replaced by compost or vermicompost.

The list presented in Table 1 is not exhaustive and could be extended through other studies (Carrión *et al.*, 2007) where for instance, disease suppressive microorganisms which have been extracted from compost are able to colonize the surface and roots of plants when applied properly (Al-Mughrabi *et al.*, 2008).

Ansorena *et al.* (2014) also argued that it is necessary to consider the limitations that bio-waste compost presents as a component of substrates and as an organic fertilizer because of its high salinity and low N concentration. Another limiting property of the compost being used as substrate may be high alkalinity. To address the latter, elemental micronized sulphur is usually added to compost (Carrión *et al.*, 2005, 2008). Also compost stability may be a key factor when compost is used as growing media to produce ornamental plants in container, so only mature compost should be utilized (Raviv, 2008, 2014).

Biochar

Biochar is another organic amendment that has the potential to be used as growing media additive and as peat substitute. Biochar is defined as a solid by-product obtained from the thermochemical conversion of biomass in an oxygen-limited environment. The process relies on capturing the off-gases from thermal decomposition of organic materials to produce heat, electricity, or biofuels (Lehmann, 2007).

'Terra preta do Indio' Amazonian soils, characterized by high levels of soil fertility, described by Sombroek (1966) started a worldwide interest to search how biochar would help to mitigate climate change (Laird, 2008; Woolf *et al.*, 2010; Montanarella & Lugato, 2013). Addition of biochar to soils can result, on average, in increased above ground productivity, crop yield, nutrient availability, microbial biomass and rhizobia nodulation among a broad range of pedo-climatic conditions. The limited number of case studies showing a negative effect of biochar on crop yield are consolidating the idea that biochar has either a null or positive effect on crop productivity (Souchie *et al.*, 2011; Alburquerque *et al.*, 2013; Biederman & Harpole,

Table 1. Growing media researches where compost and vermicompost have been used as substrate components.

Substitute type	Growing media	Raw material	% rate v/v	Plant species	Effects[a]	Reference
Compost	peat based substrate	organic fraction of urban waste	25	*Pelargonium, Salvia*	better growth	Do & Scherer, 2013
Compost	peat based substrate	sewage sludge, yard trimming and organic fraction of urban waste	25, 50	*Rosmarinus officinalis*	root collar diameter (8 to 10)% greater than control	López *et al.*, 2008
Compost	peat based substrate	sewage sludge and pruning rejects	55	*Bougainvillea*	60% increase SDW	De Lucia *et al.*, 2013
Compost	pine bark substrate	turkey litter	up to 16	*Cotoneaster dammeri*	increased (12 to 16)% CC and (17 to 30)% WHC	Tyler *et al.*, 1993
Compost	peat based substrate	green yard waste	20	*Solanum lycopersicum*	growth equal than control	Prasad & Maher, 2001
Compost	peat based substrate	nursery pruning	40	*Lantana camara, Rosmarinus officinalis*	higher overall quality	Russo *et al.*, 2016
Compost	peat based substrate	pruning from *Olea europaea, Pinus* sp. and *Picea* sp. and *Lolium perenne* clippings	20	*Lycopersicon esculentum, Cucumis melo, Lactuca sativa*	better growth	Ceglie *et al.*, 2015
Compost	peat based substrate	sludge, yard trimming and organic fraction of urban waste	20, 40	*Ceratonia siliqua, Olea europea, Quercus ilex*	RCD increased (23, 30 and 10)% respectively than control	Álvarez *et al.*, 2001
Compost	peat based substrate	sludge and urban waste	20, 40	*Pistacia lentiscus*	(509 to 730)% higher SDW than control	López *et al.*, 2003
Compost	peat based substrate	two-phase olive mill waste (71%) with olive leaves (29%) and urea (9 kg t^{-1})	25, 50	*Solanum lycopersicum, Citrullus lanatus*	better seed germination	Fernández-Hernández *et al.*, 2013
Compost	peat based substrate	sweet sorghum bagasse, pine bark and brewery slude	up to 67	*Brassica oleracea*	similar growth	Sánchez-Monedero *et al.*, 2004
Compost	peat based substrate	cow manure	10	*Solanum lycopersicum*	10% increase in roots volume	Lazcano *et al.*, 2009
Compost	peat based substrate	pruning waste	100	no plants	pH > 8, OM similar, CEC higher than control	Benito *et al.*, 2006
Compost	peat based substrate	crops waste sawdust and laying hen manure	25	*Solanum lycopersicum, Cucurbita pepo, Capsicum annuum*	better growth	Gavilanes-Terán *et al.*, 2016
Compost	peat based substrate	acacia pruning	45	*Lactuca sativa*	better growth	Brito *et al.*, 2015
Compost	peat based substrate	sewage sludge	30	*Brassica oleracea*	better growth	Perez-Murcia *et al.*, 2006
Compost	peat based substrate	cork, grape marc, olive marc and spent mushroom	100	*Cucumis sativus*	better resistance to damping-off	Trillas *et al.*, 2006
Compost	bark based substrate	organic fraction of urban waste	50	*Physocarpus opulifolius*	increased 60% SDW	Chong, 2005

Table 1. Continued.

Substitute type	Growing media	Raw material	% rate v/v	Plant species	Effects[a]	Reference
Vermicompost	peat based substrate	green and pruning wastes	30	*Petunia*	similar growth than control	Morales-Corts *et al.*, 2014
Vermicompost	peat based substrate	pig manure	30, 40	*Calendula officinalis*	more vegetative growth and flowers	Arancon *et al.*, 2005
Vermicompost	peat based substrate	chopped air-dried tomato-crop waste	75	*Calendula officinalis*	20% increase in SDW	Belda *et al.*, 2013
Vermicompost	peat based substrate	pig slurry	100	*Solanum lycopersicum*	15% increase roots volume	Lazcano *et al.*, 2009
Vermicompost	top soil	cattle manure	up to 10	*Passiflora edulis*	nursery commercial quality	Hidalgo *et al.*, 2009
Vermicompost	peat based substrate	from tomato crop waste	50	*Rosmarinus officinalis*	better growth	Mendoza-Hernández *et al.*, 2014
Vermicompost	dried sandy loam top-soil	cow manure	10	*Zinnia elegans*	better growth	Sultana *et al.*, 2015
Vermicompost	peat based substrate	pig manure	20	*Solanum lycopersicum*, *Calendula officinalis*	better growth	Bachman & Metzger, 2008
Vermicompost	peat based substrate	N/A	20	*Solanum lycopersicum*	similar emergence, growth and biomass allocation	Zaller, 2007
Vermicompost	peat based substrate	pig manure	20	*Solanum lycopersicum*	increased 12.5% fruit weight	Atiyeh *et al.*, 2000
Vermicompost	peat based substrate	cow manure	10	*Solanum lycopersicum*	60% increase in SDW	Tringovska & Dintcheva, 2012

[a]SDW: shoot dry weight; CC: container capacity; WHC: water holding capacity; RCD: root collar diameter.

2013; Carter *et al.*, 2013; Mulcahy *et al.*, 2013; Akhtar *et al.*, 2014; Thomazini *et al.*, 2015; Lima *et al.*, 2016; Olmo *et al.*, 2016).

In fact, the production of biochar from farm wastes and their application in farm soils offer multiple environmental and financial benefits (Srinivasarao *et al.*, 2013).

The priming effect concept was initially introduced by Bingeman *et al.* (1953) and may happen when biochar is added to soil. If used to describe C turnover it means an added decomposition of organic C following an inclusion of easily decomposable organic materials to the soil (Dalenberg & Jager, 1989). In the present study, the most prominent interest is related to the negative result of the priming effect of biochar because a higher retention of carbon in the substrate. No study to this effect has been found when biochar was added to peat based horticultural growing media. Nevertheless, there are several references of biochar incorporation in soil causing a negative priming effect in sandy soils which may be the most easily assimilated into the peat-based horticultural substrates (Lu *et al.*, 2014; Keith *et al.*, 2015).

Biochar has also been considered as a possible peat replacement in horticulture (Peterson & Jackson, 2014). It has shown potential as replacement for aggregates like peat moss in growing media (Sohi *et al.*, 2013). Adding biochar to growing media can result in several benefits in terms of substrate quality. Biochar generally has a high cation exchange capacity (CEC) and a high nutrient holding capacity, thereby reducing nutrient leaching. Biochar can also be considered as a source of nutrients (nitrate-N, K, Fe, Mn, and Zn) (Nemati *et al.*, 2015). This property must be taken into consideration during nutrient management planning. Most biochars are alkaline and can neutralize the acidity of a peat-based substrate, hence reducing lime requirements (Zaccheo *et al.*, 2014; Bedussi *et al.*, 2015). However, the increase of pH following a biochar application in growing media limits its application as its affects growth in plant´s germination (Buss *et al.*, 2016). In general, biochar has a low bulk density and when incorporated into a growing mix helps to reduce the risk of substrate compaction and related problems (Nemati *et al.*, 2015). Biochar can affect both water retention (Cao *et al.*, 2014) and substrate´s aeration properties depending on its particle size distribution. The incorporation of fine-textured biochar in growing media promotes water retention properties (easy and total available water) (Nemati *et al.*, 2015). Biochar particle size distribution is affected by type of biomass and the pyrolysis temperature. Choosing a biochar with the right particle size distribution is important in producing a growing mix with the desired physical properties. High-temperature biochars can bind soil-C and other nutrients on a long-term basis. In addition, higher temperature biochars have higher surface area and more micropore volumes than those of lower temperature biochars (Mukherjee & Lal, 2013).

One of the main limiting factors to the use of biochar in the growing media industry is the production of black dust during handling. Increasing the initial water content of biochar or using pelleted biochar can overcome the dust issues (Dumroese *et al.*, 2011).

It has also been reported in some phytopathological studies that biochar and its associated microorganisms have a suppressive effect on plant diseases similar to those possessed by the compost (Elad *et al.*, 2010; Elmer & Pignatello, 2011; Kolton *et al.*, 2011; Zwart & Kim, 2012; Gravel *et al.*, 2013).

Several successful propagating ornamental plant experiments have been reported where peat and some other components were replaced by biochar (see Table 2). The inclusion of biochar into substrates showed that plant´s quality and growth were similar to those from the standard peat substrates. Besides, some extra benefits were also observed in reducing nutrients and water loss, decreasing substrate bulk density, and creating a beneficial environment for microorganisms. In these experiments the peat-based substrate was substituted by biochar at a 20 to 25% average ratio (Table 2).

The wide range of raw materials to produce biochar include wood, bark and remains of coniferous (Zwart & Kim, 2012; Gravel *et al.*, 2013; Gu *et al.*, 2013; Fascella, 2015; Dispenza *et al.*, 2016) deciduous trees (Graber *et al.*, 2010; Elmer & Pignatello, 2011; Northup, 2013; De Tender *et al.*, 2016), agricultural (Dumroese *et al.*, 2011; Sharkawi *et al.*, 2014; Vaughn *et al.*, 2015a; Kim *et al.*, 2016) and gardening residues (Tian *et al.*, 2012; Nieto *et al.*, 2016) and biosolids (Méndez *et al.*, 2016). The benefits derived from the addition of biochar included improvements of morphological parameters of plants growth but also those of the physical (Kaudal *et al.*, 2015; Dumroese & Landis, 2016), chemical (Altland & Krause, 2012; Kaudal *et al.*, 2015) and biological (Elmer & Pignatello, 2011) properties of the substrate and the resistance of plants to fungal infections (Elad *et al.*, 2010; Zwart & Kim, 2012).

Carbon footprint reduction in containerized ornamental plants production

Several LCA (Life Cycle Assessment) studies have been conducted in different regions to determine which materials and activities contribute more to the GHG effect in ornamental horticulture. One of these studies assessed the material and energy inputs required to produce a *Petunia* × *hybrida* plant from initial propagation to delivery at a regional distribution centre. Impacts were expressed in terms of their contributions to the carbon footprint or global warming potential of a single finished plant in a 10-cm diameter container. Results showed that peat consumption represented 7.7% of the overall CO_2e (carbon dioxide equivalent) emissions (Koeser *et al.*, 2014).

Two LCA studies conducted in Italy (De Lucia, 2013; Vecchietti *et al.*, 2013) considered compost as growing media substitute. The use of different rates of sewage sludge compost in the preparation of growing media for potted *Bougainvillea* was evaluated to assess its efficiency for the replacement of peat and to quantify the environmental impact of such alternative substrates. The data from LCA showed that the addition of compost reduced the environmental impact of the plant nursery. Specifically, the use of compost reduced ODP (ozone layer depletion index) by 23-42% and also the primary non-renewable energy consumption index by 40-80% when compost was added to the mixture (as 25%-70% of compost inclusion respectively in both indexes).

Altieri & Nicholls (2012) and Martínez-Blanco *et al.* (2013) reported the positive effects of compost application as nutrient supply and carbon sequestration and also opined that the benefits were quantifiable, and tools for their consideration with LCA were available. Regarding the supply of plant nutrients, between 5 and 60% of the N applied with compost was mineralized, depending on the time frame considered. Figures range between 35 and 100% for P and between 75 and 100% for K. Carbon sequestration rates have shown to be higher in the short term (up to 40% of the applied C) and decreasing to 2–16% over a 100-year period (Martínez-Blanco *et al.*, 2013). Hence, those benefits should be regularly included in LCA studies, although their quantification needs to be improved.

Russo *et al.* (2008), in another LCA study on cyclamen in container production reported that as the peat is a non-recyclable organic material, it can find a substitute in the green composts obtained by the treatment of municipal garden green wastes and pruning wastes.

Finally, another study, conducted in Germany reported the amount of reduced GHG emissions by substitution of peat with biochar. This substitution could

Table 2. Growing media researches where biochar has been used as substrate component.

Substitute type	Growing media	Raw material	% rate v/v	Plant specie	Effects[a]	Reference
biochar	peat based substrate	*Pinus* sp wood	5, 10, 15, 20, 25 and 30	*Gomphrena 'Fireworks'*	similar growth as control	Gu *et al.*, 2013
biochar	peat based substrate	*Pinus* sp wood	5, 10, 20	*Acer rubrum, Quercus rubra*	alleviate disease progression and physiological stress caused by *Phytophthora* canker pathogens	Zwart & Kim, 2012
biochar	peat based substrate	*Abies alba, Larix decidua, Picea excels, Pinus nigra*	60	*Euphorbia* × *lomi*	better growth	Dispenza *et al.*, 2016
biochar	peat based substrate	*Quercus ilex* wood	3% w/w	*Fragaria* × *ananassa*	160% increase in SDW	De Tender *et al.*, 2016
biochar	peat based substrate	hardwood	20, 30, 40	*Calendula officinalis, Petunia* × *hybrida, Impatiens*	SDW similar or greater than control	Northup, 2013
biochar	peat based substrate	hardwood dust	10	*Asparagus*	increased arbuscular mycorrhizal root colonization	Elmer & Pignatello, 2011
biochar	peat based substrate	hardwood pellets and pelletized wheat straw	10,15	*Calendula officinalis*	increased plant height	Vaughn *et al.*, 2013
biochar	peat based substrate	*Abies balsamea, Picea glauca* and *Picea mariana* softwood bark	50	*Pelargonium hortorum*	similar growth as control	Gravel *et al.*, 2013
biochar	peat based substrate	crushed wooden boxes	25, 50, 75	*Helianthus annuus*	similar growth as control	Steiner & Harttung, 2014
biochar	peat based substrate	pruning residue	50, 75	*Lactuca sativa*	better growth as control	Nieto *et al.*, 2016
biochar	peat based substrate	green waste	50	*Calathea rotundifola* cv. Fasciata	22% total biomass increase	Tian *et al.*, 2012
biochar	peat based substrate	biomass	1, 5, 10	no plants	moderation of extreme fluctuations of nitrate levels	Altland & Locke, 2012
biochar	peat based substrate	agricultural or forestry residues	25	no plants	enhanced hydraulic conductivity and greater water availability	Dumroese *et al.*, 2011
biochar	peat based substrate	biosolids	10	*Lactuca sativa*	better growth as control	Méndez *et al.*, 2016
biochar +digestate	peat based substrate	wood pellets, pelletized wheat straw and field pennycress presscake + potato anaerobic digestate	25	*Solanum lycopersicum, Calendula officinalis*	increased growth of tomato plants and equal marigold as compared to control	Vaughn *et al.*, 2015a

Table 2. Continued.

Substitute type	Growing media	Raw material	% rate v/v	Plant specie	Effects[a]	Reference
biochar	peat based substrate	conifers wood	60	*Euphorbia* × *lomi*	higher stem diameter, leaves area, root length and number of flowers than control	Fascella, 2015
biochar	coco fiber	forestry and gardening waste	10	*Calendula officinalis, Petunia* × *hybrid*	better growth as control	Fornes *et al*., 2013
biochar	coconut fiber and tuff	*Citrus* wood	5	*Capsicum annuum, Solanum lycopersicum*	better pepper growth and enhanced tomato plant height and leaf size.	Graber *et al*., 2010
biochar	coconut fiber-tuff	*Citrus* wood	1, 3, 5% w/w	*Capsicum annuum, Solanum lycopersicum*	resistance against two foliar fungal pathogens (*B. cinerea* and *L. taurica*)	Elad *et al*., 2010
biochar	coir peat	biosolids and greenwaste	up to 60	no plants	similar physical and chemical benefits than control	Kaudal *et al*., 2016
biochar	coir peat+pine bark compost	biosolids and greenwaste	20, 40, 60	no plants	desirable physical properties such as high water holding capacity, low bulk density, air filled pore space and high surface area	Kaudal *et al*., 2015
biochar	rice husk	rice husk	25	*Cucumis sativus*	better growth as control	Sharkawi *et al*., 2014
biochar	coir dust, perlite and vermiculite	rice husk	5% w/w	*Brassica oleracea*	150% increase in SDW	Kim *et al*., 2016

[a] SDW: shoot dry weight.

avoid emissions of up to 4.5 Mg of CO_2e by each Mg of peat substituted (2.8 Mg CO_2/Mg by biochar inclusion plus 1.7 Mg CO_2 Mg by peat substitution) (Steiner & Harttung, 2014).

In the studies and experiments mentioned above, peat based substrates were substituted at a 30-35% average ratio by compost and vermicompost and 20-25% by biochar. We have calculated reduced GHG emissions by considering these substitution ratios as well as average bulk density levels of peat based growing media, compost/vermicompost and biochar. We have taken into account that every year about 11 Tg of peat are consumed in horticulture. If 20% of worldwide peat used in horticulture would be in containers production, about 3 Tg CO_2e will be the C potential storage per year that this container productive sector will be able to generate when peat based growing media has been substituted as above mentioned.

Research gaps

Globally, there is a lack of information about the total volume of materials used in growing media in countries with an important production in South and North America, Australia and Southeast Asia (Carlile, 2008; Schmilewski, 2017).

Research on how to use compost and vermicompost as partial replacement of peat based growing media to produce ornamental plants has been more addressed by research studies (Raviv *et al.*, 1986; Edwards & Burrows, 1988; Carrión *et al.*, 2007) than the use of biochar. There are also a number of research gaps about how to combine either compost or vermicompost with biochar to substitute peat in this ornamental horticulture industry. That is why we have tried below to identify potential research projects able to get answers to the pending questions.

Assuming that biochar is a panacea without strong scientific evidence and credible data, may aggravate controversies and dilemmas (Perry, 2011; Mukherjee & Lal, 2013; Lal, 2015). This is a key point considering biochar's characteristics variability due to raw materials and production systems (Lorenz & Lal, 2014). For instance, in some studies identical biochars produced different results with different plant species (Vaughn *et al.*, 2015c). Some but not all biochars have been shown to improve water retention and increase overall plant growth in sand-based rooting media. Impact of biochar on improvement of water retention and increase overall plant growth in sand-based root zones may happen with some but not with all biochars (Vaughn *et al.*, 2015b). Also, it would be necessary to identify from which tree species or type of waste material biochar would be most desirable for use in horticultural potting substrates (Vaughn *et al.*, 2015a).

Results from some biochar studies begin to provide evidence of mitigation strategies, which can be implemented in container plant production to help growers benefit from C offset programs, adapt to future legislation, and improve the environmental impact from container plant production without negatively affecting crop growth (Marble *et al.*, 2012). So, more product carbon footprint analyses are necessary to map out the climate impact in different horticultural production systems (Soode *et al.*, 2015). It would be also useful to know what CO_2 percentage could ornamental horticulture represent respect to global horticulture production.

Additionally, there are some experiments that demonstrate the synergy of combining biochar with compost in soil (Schmidt *et al.*, 2014). This positive association is caused mainly because the combination of both materials improved its fertility, not only in a short time span, but also on a medium and long term basis (Fischer & Glaser, 2012). Compost and vermicompost have shown a good synergy with biochar, but literature about this combination in ornamental horticulture is rather scanty. Just one study using vermicompost and biochar to produce ornamentals in containers was found (Alvarez *et al.*, 2017). Both materials were mixed with no prior composting. A complete set of 24 combinations, where a peat-based substrate was partially replaced by 0 to 50% of dairy manure vermicompost and 0 to 12% of biochar produced by pyrolysis of *Pinus monticola* wood at high temperature (600 to 800 ºC). Better *Petunia hybrida* and *Pelargonium peltatum* plant growth and flowering was obtained in some of the mixtures of biochar/vermicompost with no more than 30% of vermicompost content than in the control group. Even if most plant responses are related to morphological parameters it would be interesting to also test physiological parameters as they may provide results regarding plants growth after transplanting into soil (Alvarez *et al.*, 2018).

There are some other studies where that kind of mix was applied to soil and assessed plant or soil responses (Schulz & Glaser, 2012; Ngo *et al.*, 2013; Rodríguez-Vila *et al.*, 2014). So, more experience combining compost or vermicompost with biochar to substitute peat-based substrates in ornamental horticulture should be promoted to learn whether their synergy would be interesting for the industry and with the objective of carbon sequestration. There are a number of publications where biochar was added to other organic materials to be co-composted or composted together and a synergy was evident during this combined process enhancing the final compost produced. Even if there is no evidence yet of the proven results when using this kind of final product to replace peat in ornamental production, these trends are briefly discussed herein because it would be pertinent to research this subject (Dias *et al.*, 2010; Jindo *et al.*, 2012, 2016; Schulz *et al.*, 2013; Antonius *et al.*, 2015; Barthod *et al.*, 2016; Malińska *et al.*, 2016).

The ornamental containerized plant sector needs to develop a better understanding of plant nutrient requirements, better technology to assess root zone conditions, and better fertilizers or practices that would be able to match ornamental plant nutrient requirements during the growing season in containers. With a satisfactorily resolution of this sector, Majsztrik *et al.* (2011) and Raviv (2013) concluded that horticulture can provide ecological services such as efficient and long-term carbon sequestration, while restoring soil fertility through the use of organic amendments. In this context evaluating how to include compost, vermicompost and biochar (and their mixes) may minimize leaching of nutrients from containers due to irrigation. This subject is also a researchable priority.

As Nemati *et al.* (2015) commented, compost, vermicompost and biochar are still not a standardized product, and its properties may differ depending on the source or the production process. The growing media industry cannot accept these variations and requires a high quality, homogenous, and consistent components. Therefore, it is important to launch a standardization program to certify those materials which meet quality standards for use in the growing media industry. In this sense, it is important to bridge the gap between research findings and commercial production of ornamental plants by assessing the experimental results at a commercial scale (Vaughn *et al.*, 2015c; Derrien *et al.*, 2016).

Economically, biochar has a greater potential to replace aggregates than peat in growing media mainly due to the high cost of these aggregates compared with that of the peat. Additional research is needed to evaluate the impact of biochar on growth and development of plants.

Conclusions

The use of organic materials as compost, vermicompost, and biochar as peat substitutes in the ornamental containerized bedding plant production, is an interesting biotic strategy to store carbon in garden soil. In the case of biochar the stored C could be maintained for centuries improving the life cycle analysis of this process.

Several studies have produced interesting results, but additional research is needed to evaluate those materials and how to combine them as compost-biochar or vermicompost-biochar which may produce similar or better plants while also similarly or better support the transplanting process.

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Vermicompost and Biochar as growing media replacement for ornamental plant production

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Vermicompost and biochar substrates can reduce nutrients leachates on containerized ornamental plant production

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Vermicompost and biochar substrates can reduce nutrients leachates on containerized ornamental plant production

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ABSTRACT

Containerized ornamental plant production is facing several environmental challenges. One of them is to replace the widely used, but with questionable sustainability, peat based substrates and another is to avoid water contamination by chemicals leaching from the nursery. Therefore, as have been verified that petunia and pelargonium plants can be produced in peat-based growing media partially replaced by vermicompost (*V*) and biochar (*B*) without decreasing commercial quality, this study has focused on analyzing the leachate from a standard peat-based substrate as a control, used for producing these two ornamental species, and those from the same substrate to which different proportions in volume of *V* (10 % and 20 %) and *B* (4 % and 12 %) have been added. It has been found that the amount of nitrogen leached from the mixed substrates was reduced compared to the control one in both species (on average 37 %). Nitrogen was leached mainly as nitrate-nitrogen (89 % in *Petunia* and 97 % in *Pelargonium*). In *Petunia* phosphorous leaching was also decreased (30 %) for the treatment with 10 % *V* and 4 % *B*, while potassium leaching in substrate containing 20 % *V* and 12 % *B* increased by 100 %. Our results show that these two organic materials tested (*V* and *B*) can help producers to reduce the use of peat and chemical fertilizers as well as the risk of contamination by chemicals, mainly nitrate.

Key words: ***Petunia hybrida, Pelargonium peltatum, peat replacement, water contamination, nitrate, phosphate***

INTRODUCTION

Containerized ornamental plants production has increased all over the world (AIPH, 2017). Growers have to face several environmental challenges both to compile legal requirements and the increasing environmental demands of their customers. We can mention three obstacles on which the producer will have sooner or later to make decisions about.

First, the use of peat as growing media is increasingly weighed. Around 10 to 11 million kg of this material are used annually in the world for horticultural production (US, 2016). Since peat is considered a non-renewable resource and its use is questioned by the drainage of peatlands (Keddy, 2010). In the frame of the circular economy there is a growing demand to use renewable materials, mainly from the recycling of organic wastes and by-products. Vermicompost (*V*) and biochar (*B*) are good candidates to substitute peat as growing media, since it has been proven that, used in the right proportions, they do not reduce, even can improve, the commercial quality of the produced plants (Alvarez et al., 2017, 2018). Vermicompost is a product derived from the accelerated biological degradation of organic wastes by earthworms and microorganisms. Biochar is a by-product of the C-negative pyrolysis technology for bio-energy production from organic materials.

Second, there is increasing awareness of the need to mitigate the effects of climate change. The use of recycled materials and alternative energies to fossil fuels are often the main changes that the ornamental plant production industry introduces when it decides to study and maintain a strategy to track the carbon footprint of its products (Barrett et al., 2016).

Finally, due to the peculiarities of this type of containerized ornamental plants production (Ruter, 1993), irrigation and fertilization management should be adequate to avoid nutrients leaching to public waters adjacent to the area of the production facilities and their eventual contamination (Cabrera, 1997, Majsztrik et al., 2011). Actually, in Europe and the United States there is an increasing pressure to reduce the leachates of horticultural crops for environmental reasons (Guimera et al., 1995). Nitrate, ammonium and phosphates are the ions that are considered the most problematic irrigation leachates (Mueller et al., 1995) due to their effect in surface waters and impact in public health (Agegnehu et al., 2017).

Our hypothesis is that the inclusion of these two new materials, biochar and vermicompost, in the peat based growing media could reduce the leaching of nutrients while maintaining an adequate plant quality.

Manuscript main objective: in this study the leaching of nitrogen and other nutrients from peat based blends including biochar and vermicompost was assessed in comparison with usual fertilized peat substrates.

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MATERIAL AND METHODS

Plant material and experimental design

Two ornamental species very much worldwide used were utilized, *Petunia x hybrida* cv. Dreams Neon and *Pelargonium peltatum* cv. Summer Showers. These species were also chosen for their different nutrients needs and rusticity as well as on their salt tolerance, being *Petunia* more tolerant than *Pelargonium* (Monk & Wiebe, 1961; Do & Scherer, 2013), since vermicompost (*V)* and biochar (*B)* could modify mineral nutrients availability, electrical conductivity and pH (Alvarez et al., 2017).

Commercial products available in the market were used to make up the growing media, biochar (*B*), vermicompost (*V*) and a peat-based substrate (*S*). The biochar is called Soil Reef Pure 02 (Biochar Solutions Inc., Co, USA) and was produced by high temperature pyrolysis, 600 to 800 ºC, of *Pinus monticola* wood. The vermicompost is named Black Diamond Vermicompost (Black Diamond Vermicompost, Ca, USA) and was produced by pre-composting for two weeks the solid fraction of bovine manure using an aerated composting system, then submitted to a vermicompost process for a period of 70 to 80 days. These two renewable organic materials (*B* and *V*) were used to partially replace a peat-based control substrate (*S*) called Farfard 3B mixture (SunGro Horticulture Distribution Inc., USA). This peat-based substrate is composed by Canadian *Sphagnum* peat moss, pine bark, perlite, vermiculite, dolomitic limestone, and a wetting agent, at 6:4:2:1 Peat:Bark:Perlite:Vermiculite volume ratio. Farfard 3B received a slow release fertilizer (Scotts Osmocote Plus 15-9-12, 5-6 months release at 21°C, at a dosage of 5.9 g L^{-1}). An overview of the main characteristics of these components, and more details appear in table 1 at Alvarez et al. (2017).

Three growing media (mixes) were prepared with the following volume fractions (*S*:*V*:*B*): 100:00:00, 86:10:04 and 68:20:12, being, respectively, the control treatment and two treatments containing a slight and a moderate peat-based substrate replacement. The last two treatments were selected based on a previous study when 23 different mixes were compared with *S* (i.e. *S* = 100:00:00 treatment), and according to the good plant growth and flowering obtained (Alvarez et al., 2017). Then, bulk density (*Db*), water holding capacity (*WHC*), total porosity (*Pt*) and air space (*As*) were determined at the beginning of the experiment following the procedures for determining physical properties of horticultural substrates using the NCSU porometer (Fonteno & Bilderback, 1993). Soluble nutrients, pH and electric conductivity (EC) were determined in aqueous extracts (1:6 volume fraction) taken from fresh mixtures samples in advance of plants cultivation: nitrate and ammonium by spectrophotometry in a flow autoanalyser (AA III, Bran + Luebbe, Norderstedt, Germany) (Ansorena Miner, 1994); potassium, sulfate and phosphate by ICP-OES (Dahlquist & Knoll, 1978); EC and pH by a pH-meter/conductimeter (Acumet® Ap85, USA) (Ansorena Miner, 1994).

Petunia and *Pelargonium* seeds were germinated in 100 plug trays (21.8 cm^3/cell) and was added two seeds per cell. After germination, just one seedling was kept. Trays were placed in a glasshouse for 40 days at 24 °C and 54 % average temperature and relative humidity, respectively under a climate control system in the greenhouse. Watering was done with an automatic micro sprinkler irrigation system between dawn and dusk. Nozzles were irrigating at 1.8 L/h during 15 seconds every 20 min, with 2 m diameter and 1 meter overlap. After that, thirty seedlings were randomly obtained, transplanted into 800 cm^3 plastic containers and moved to a glass covered greenhouse (average temperature 20 °C and average humidity 29 % also under a climate control system in the glasshouse) for 68 days until the market size was reached. Standard propagation protocols for these species were followed. The experimental design was a completely randomized design with two replicas. Each replica consisted of 5 plants per species and treatment randomly distributed (5 plants x 3 treatments x 2 species = 30 plants per replica). The two replicas were placed on separate benches (2 replicas x 15 plants = 60 plants). Plants were rotated periodically to minimize variation in microclimatic conditions. Containers were watered manually as needed with distilled water. The water was added to each pot gradually by using a slight volume every time ($\leq 10\ cm^3$) and waiting for a few minutes before adding next volume. As soon as a water droplet appeared at the bottom of the pot no more water was added. These few water droplets leached from each pot were taken back to the pot. Therefore, water was gradually added trying to avoid leaching and to keep substrate to field capacity.

Plant growth, leaching parameters and data analysis

The parameters evaluated were shoot dry weight (SDW) of plants, and containers leachates volume and nutrient contents. At the end of the growing period and before measuring shoot dry weight (SDW) of plants, containers leachates were collected during five consecutive days after receiving a daily watering of 200 cm^3. In order to collect the leachate, both a plastic mesh and a plastic cuvette were placed under each container. For every sampling date, the substrate was moistened to field capacity, as described before, one day before to collect the samples. Collected volume was measured and a sample was taken for subsequent nutrient analysis of nitrate-nitrogen ($N\text{-}NO_3^-$), nitrite-nitrogen ($N\text{-}NO_2^-$), ammonium-nitrogen ($N\text{-}NH_4^+$), phosphate-phosphorous ($P\text{-}PO_4^{-3}$), total P, sulfate (SO_4^{-2}). The total nitrogen was calculated as the sum of nitrate-, nitrite-, and ammonium-nitrogen. The nutrient contents (mg) collected in the leachates were calculated by multiplying the concentration (mg L^{-1}) by the collected volume (L). Nutrient analysis was performed by means of standard methods using a multiparameter photometer (HI 83200, Hanna Instruments®, Italy).

At the end of the growth period SDW and number of flowers were recorded in *Petunia* and *Pelargonium* plants. In pelargonium number of open inflorescences and inflorescence-buds were also counted. Shoot dry weight was obtained after oven-drying at 55 °C for 72 h. For SDW and inflorescences, one-way analysis of variance (SPSS Statistics 17.0) were carried out to determine statistically significant differences between treatments, being the treatment a fixed effect. While for leachate nutrient concentrations and nutrient contents repeated measured ANOVA were carried out, since nutrient concentration in the leachate on a specific day depends on the concentration obtained in previous days. Significant differences were established at $p = 0.05$. To evaluate the among treatments comparisons, Tukey-HSD or T3-Dunnett tests were used in order to differentiate within homogeneous groups, according to variance homoscedasticity. In addition, correlation and regression analysis were performed in order to establish the underlying relationships between treatments and measured parameters.

RESULTS AND DISCUSSION

Physical characteristics of the substrates and plant growth

The physical properties at the beginning of the experiment of peat-based substrate (*S*), and the two different mixtures studied are shown in Table 1. *Pt* and *As* in all mixtures lied within the suggested optimum ranges, 68 to 88 % and 6 to 13 %, respectively. *WHC* was always slightly above the recommended range 45 to 65 %, while *Db* was also slightly above the recommended range (100 to 140 kg m^{-3}), except for control. All the above met the recommendations made by Bilderback et al. (2005) and Yeager (1997).

Figure 1 shows accumulated plants biomass and number of flowers per plant for the two ornamental species grown in the three different treatments. *Petunia'*s SDW and flowering were significantly higher in mixture 86:10:04 compared with control. Treatment 86:10:04 grown up to 37 % and produced 43 % more flowers than the standard peat based substrate. Mix 68:20:12 produced 30 % more flowers than control. In the case of *Pelargonium*, SDW was similar in all treatments, but flowering in mix 86:10:04 significantly and positively differed from the control, producing up to 108 % more flowers. In regard to physical and physico-chemical characteristics of these three substrates, only bulk density (D_b) was affected by the addition of *V* and *B*, being the heaviest mixture (68:20:12) only a 10 % heavier than the control one. The addition of *V* to peat substrates usually increase D_b (Mupondi et al., 2014; Álvarez et al., 2017), but in this study, taking into account the proportions of *V* used, it does not seem to have negatively affected the plant growth and nursery management. In respect of plant growth and flower production, our results clearly showed that *Petunia* and *Pelargonium* growth and flowering status was enhanced with the inclusion of *B* and *V* in peat based substrate in slight or moderate proportions. These results are aligned with other species (Graber et al., 2010; Tian et al., 2012; Mulcahy et al., 2013). For instance, Graber et al. (2010) found an increase in pepper canopy dry weight and flowering by the addition of biochar to a coconut fiber:tuff mix; Tian et al. (2012) obtained similar results growing *Calathea rotundifolia* plants in 50 % green waste pyrolyzed biochar added to a peat medium, compared to 100 % peat; and an improvement in tomato plant height in growing medium amended with wood pyrolyzed biochar (1 to 5 %, weight fraction).

Leachate properties

On average, *Pelargonium's* leachate volume per pot and date (50.6 cm^3) was 47 % lower than *Petunia*'s (74.4 cm^3). For both species, neither the effect of treatment ($p \geq 0.107$) nor treatment x date interaction ($p \geq 0.561$) were significant (Figure 2). However, the sampling date was significant ($p \leq 0.005$): for *Pelargonium* it ranged from 33.9 cm^3 (day 3) to 68.4 cm^3 (day 1), whereas for *Petunia* it did from 40.5 cm^3 (day 1) to 107.7 cm^3 (day 5), but without following a defined pattern between consecutive days.

For both species, collected leachates did not show significant differences in pH between sampling dates ($p \geq 0.165$) nor for treatment x date interaction ($p \geq 0.405$), but the effect of treatment was significant ($p <0.001$). The ranking between treatments was 100:00:00 < 86:10:04 < 68:20:12, with values around neutral, slightly lower for *Pelargonium* (6.5 < 7.1 < 7.5, respectively) than for *Petunia* (7.0 < 7.6 < 7.9, respectively). The increase in pH was well correlated to both components added to peat-based substrate. In *Petunia,* pH was significantly related to *B* (R^2 = 0.72, $p < 0.01$, n = 30) and to *V* (R^2 = 0.79, $p < 0.01$, n = 30). Also in *Pelargonium,* pH was related to *B* (R^2 = 0.72, $p < 0.01$, n = 30) and *V* (R^2 = 0.69, $p < 0.01$, n = 30).

EC was higher in *Pelargonium* (4.3 ± 0.2 dS m^{-1}) than in *Petunia* (1.9 ± 0.1 dS m^{-1}), with no significant differences between sampling dates ($p \geq 0.155$) nor between treatments for *Pelargonium* ($p = 0.415$). However, the treatment effect was significant for *Petunia* ($p = 0.012$). The mean values for *Petunia* were 1.7, 1.9 and 2.1 dS m^{-1} for 68:20:12, 86:10:04 and 100:00:00, respectively, being significant the differences between the two most extreme treatments.

The treatment x date interaction was not significant ($0.063 < p < 0.873$) in mineral nutrients concentrations and contents in leachate. Leachate´s concentration of sulfate ions (SO_4^{-2}) did not differ significantly between treatments for either species ($p \geq 0.884$), but there was difference between species, resulting in a 27.6 % higher for *Pelargonium* 401 mg L^{-1} than for *Petunia* 314 mg L^{-1}. However, sampling date was significant ($p \leq 0.038$) for sulfate ions, as concentration decreased from the first to the last date: 446 to 343 mg L^{-1} for *Pelargonium* and 363 to 240 mg L^{-1} for *Petunia*. Total sulfur´s amount (S, contained in sulfate ions, i.e. S-SO_4^{-2}) per pot, as the sum of the five days sampled, averaged 35 mg for *Pelargonium* and 38 mg for *Petunia*.

Table 2 shows N, P and K leachates concentration values. N concentration in leachates was reduced in the mixed substrates compared to the control one in both species, while K concentration increased. In the case of N, concentration decreased 18 to 22 % in *Petunia*, and 17 to 40 % in *Pelargonium*. Whereas for K, the increments were 97 % in *Petunia,* but only significant for the 68:20:12 treatment, and 29 to 53 % in *Pelargonium.* In *Petunia* phosphate-P form represented 46 % of the total P, whereas for *Pelargonium* it was 61 %. Regarding N, in *Petunia*, 89 % corresponded to nitrate-N, 10.9 % to ammonium-N and the remaining 0.1 % to nitrite-N. In *Pelargonium*, respective percentages were 97 %, 2.9 % and 0.1 %.

Figure 3 shows N, P and K total amount leached per pot during the five sampling days. The amount of nitrogen leached from the mixed substrates was reduced compared to the control one in both species (32 to 43 % in *Petunia* and 26 to 47 % in *Pelargonium*). These reductions were greater than the 14 and 32% reduction that could be attributed to the dilution of the control substrate in the mixtures 86:10:04 and 68:20:12 respectively. In *Petunia* phosphorous decreased (30 %) for the 86:10:04 treatment, while potassium in 68:20:12 treatment increased by 100 %. Nutrients leached amount measurement related to the inorganic fertilizer added to the peat-based substrate and how much was a contribution of either *V* or *B* was not performed. In particular, *V* contained a large amount of N, P and S, while *B* of K, P and S. For instance, in the case of N, the peat-based substrate together with the 5.9 g/L of inorganic fertilizer added implied 892 mg/L of soluble N (1857 mg/L of total N) in that substrate, while *V* contained 408 mg/L of soluble N, and 3799 mg/L of total N. Therefore, *V* contained less soluble N but more total N to be released slowly over time. Anyway, it is clear that there has been an interaction in the nutrient retention capacity between the different components of the substrate mixture, since: i) the amount of added inorganic fertilizer was reduced, regarding control treatment, 14% for 86:10:04 and 32% for 68:20:12; ii) water leached by alternative treatments, regarding control, presented, in general, a lower concentration of N, greater than K and equal to P and S; iii) in terms of total amount of nutrients leached (Fig. 3) percentage reduction of N and P in the two alternative treatments was greater than the reduction of added fertilizer. In any case, as SDW and the number of flowers were not decreased, the overall response of the two mixtures containing *V* and *B* seems to be environmentally more attractive than peat based substrate to which soluble inorganic fertilizer need to be added, at least for nitrates and phosphates.

Taking into account the correlation analysis performed between leachate parameters, pH and EC, it can be highlighted that: a) for both species, the total amount of nutrients in each leached sample (N, P, K, S) were positively correlated between themselves ($0.49 < r < 0.89$, $p < 0.001$, $48 < n < 75$); b) for both species, N content and N concentration were negatively correlated with pH ($-0.67 < r < -0.43$, $p < 0.025$, $n = 30$); for *Pelargonium*, EC was positively correlated with N, K and S concentrations and content ($0.44 < r < 0.79$, $p < 0.023$, $n = 30$).

Regarding leachates, the slight pH increase (an increment of only about 1.0) when *V* and *B* were added to the standard peat based growing media shows the capacity of *B* and *V* to serve as a liming agent when added to a peat-based substrate, in addition to their effects on the physical properties (Northup, 2013).

In reference to nutrient content in leachates, it was observed that less quantity of N, K and S has been leached in petunia compared to pelargonium. This fact also coincides with a remarkable greater production of flowers in the former species. In addition, the lower N and S concentrations in the leachates from *Petunia* (and therefore lower nitrate-N and sulfate-S) may be related to the higher pH compared to *Pelargonium*. Likewise, the higher N, K and S concentrations in the *Pelargonium* leachates compared to *Petunia*, may have influenced the positive relationship between these nutrients and EC in the former species.

The fact is that N concentration (Table 2) and N content (Figure 3) in leachates significantly decreased for both species as *V* and *B* increased, which could be due to nitrate retained to the biochar-vermicompost ensemble and more slowly released (Altland & Locke, 2013; Kammann & Clough, 2014).

On the other hand, the increase of potassium concentration in leachates (and content for *Petunia*) as the ratio of biochar applied to the mixtures was also observed in Malińska et al. (2016), in which it was noted that biochar could be a significant source of K and should be accounted for in fertility programs (Altland & Locke, 2013). It is not considered necessary to establish a health-based guideline value for potassium in drinking-water. Although potassium may cause some health effects in susceptible individuals, potassium intake from drinking-water is well below the level at which adverse health effects may occur (WHO, 2009). *Petunia*´s leachates, even if higher in volume, had less N and K concentration and content than *Pelargonium*´s probably due a minor nutrients need of last specie to grow and produce flowers (Karras et al. 2016). Therefore, the species grown in the pot can also affect the leachate mineral composition.

This study has verified a partial reduction of nitrogen (mainly nitrate) in both species, and slightly P in Petunia, leached from the containers as consequence of the biochar-vermicompost inclusion in the selected mixtures additional to the reduction due to the lower ratio of the control substrate in the mixtures. Also, biochar addition could be a significant source of potassium in growing media and may be considered in fertility programs. So, first section of our hypothesis was partially demonstrated.

Obtaining commercial quality plants with similar or even greater growth and flowering than control substrate has served to evidence our second section of our hypothesis that renewable materials can be used for the production of these containerized ornamental plants.

Finally, as biochar produced from high temperature pyrolysis had more recalcitrant character for carbon sequestration and was able to store carbon in soil for longer periods of time (Jindo et al., 2014), so the third section of our hypothesis - climate change mitigation by reducing carbon foot print in this commercial sector - has also been positively addressed.

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TABLES AND FIGURES

Table 1: Physical properties, mean (SE), of growth media (treatments) used in the experiment.

	Db	*WHC*	*Pt*	*As*
S:V:B	**kg dm^{-3}**	**%**	**% v/v**	**% v/v**
100:00:00	0.140 (0.03) a	70.1 (0.6) a	80.1 (0.2) a	10.3 (0.9) a
86:10:04	0.143 (0.05) a	71.5 (0.7) a	80.3 (0.6) a	8.7 (1.2) a
68:20:12	0.154 (0.02) b	72.2 (0.6) a	80.7 (0.8) a	8.2 (0.3) a
p	0.02	0.12	0.87	0.30

Db = bulk density; *WHC* = water holding capacity; *Pt* = total porosity; *As* = air space.
S = peat-based substrate, *V* = vermicompost, *B* = Biochar. Control, 100:00:00. Volume fraction (%).
p = significance level. Different letters in numerical columns differ at $p \leq 0.05$ (Tukey-HSD test for *Va*, *Pt* and *As*; T3-Dunnett test for *Db*). Columbus. OSU. 2016.

Table 2: Concentration, mean (SE), of N, P and K in the leachate collected from each pot for the different treatments and sampling dates. Huelva, ETSI, 2017.

(mg L^{-1})	***Petunia***			***Pelargonium***		
	N	**P**	**K**	**N**	**P**	**K**
Treatment						
100:00:00	52.1 (3.8) b	23.1 (0.7) a	46.5 (4.4) a	247 (14) c	18.2 (1.3) a	208 (22) a
86:10:04	40.8 (4.0) a	21.6 (0.6) a	47.1 (3.6) a	205 (13) b	19.0 (0.9) a	269 (21) ab
68:20:12	42.9 (2.5) ab	24.4 (0.9) a	91.6 (5.6) b	148 (9) a	18.5 (1.2) a	318 (28) b
p	0.031	0.052	0.034	0.016	0.958	0.034
Date						
1st day	55.7 (5.5) c	21.5 (1.1) a	71.9 (9.3) b	246 (28) b	19.7 (1.5) a	356 (32) b
2nd day	53.1 (3.8) bc	22.7 (0.7) a	69.7 (8.0) ab	230 (21) ab	19.3 (1.4) a	290 (21) b
3rd day	48.2 (3.6) bc	23.1 (1.2) a	66.5 (9.8) ab	190 (14) ab	20.1 (1.7) a	229 (27) b
4th day	39.6 (4.0) ab	23.3 (0.6) a	56.9 (6.9) ab	180 (11) a	18.1 (1.1) a	203 (22) ab
5th day	30.8 (4.3) a	24.5 (1.4) a	48.9 (6.9) a	173 (12) a	15.6 (1.4) a	186 (23) a
p	0.006	0.131	0.039	0.013	0.056	0.003

p = significance level at 0.05. Different letters in numerical columns differ at $p \leq 0.05$ (Tukey-HSD test). A

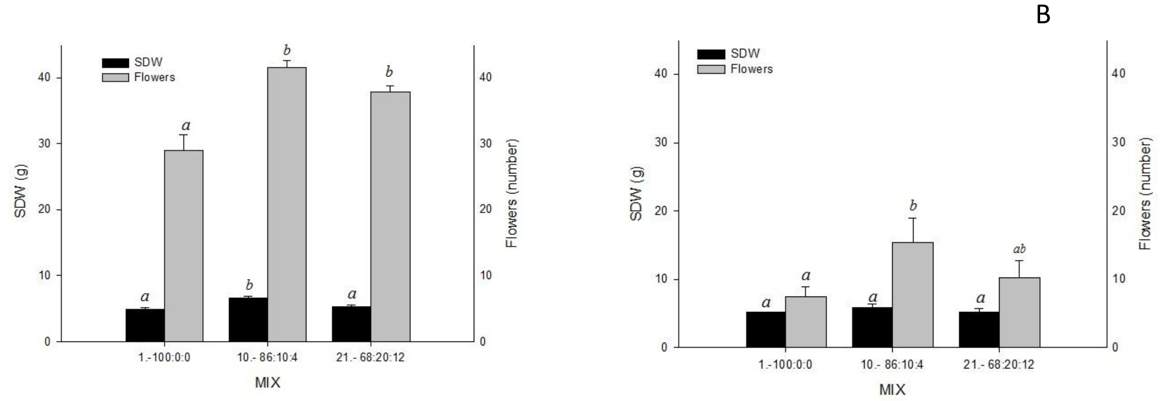

Figure 1: Shoot dry weight (SDW, g) and flower production number of petunia (A) and pelargonium (B) grown in mixtures with different proportions of peat-based substrate (*S*), vermicompost (*V*) and biochar (*B*) (*S*:*V*:*B*). Letters show significant differences between substrates studied ($p < 0.05$). (Tukey-HSD test for SDW both species, and for Flowers in *Petunia*; T3-Dunnet test for Flowers in *Pelargonium*). Columbus, OSU, 2016.

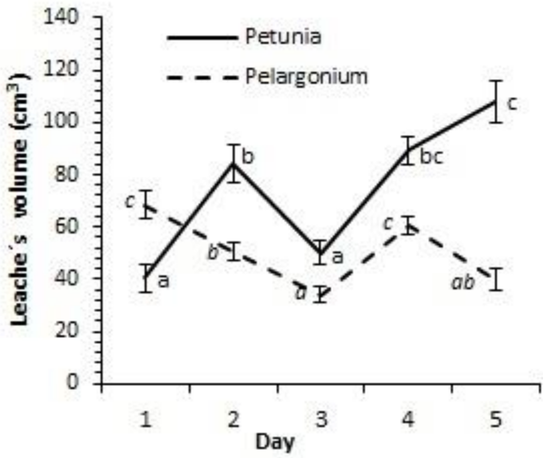

Figure 2: Leachate´s volume (cm^3) of petunia and geranium grown in mixtures with different proportions of peat-based substrate (*S*), vermicompost (*V*) and biochar (*B*), (*S*:*V*:*B*). For each species letters show significant differences among sampling dates ($p < 0.05$). Huelva, ETSI, 2017.

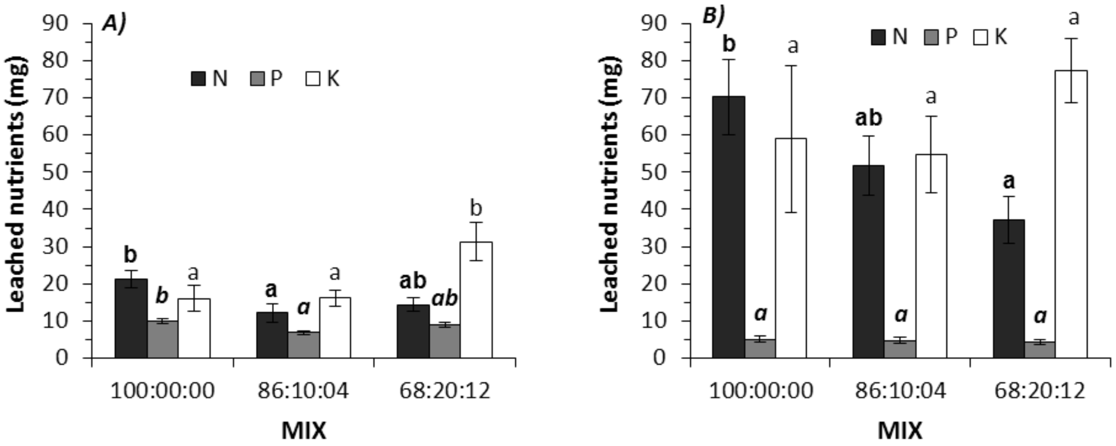

Figure 3: Total amount of nutrient leached by containers taken into account the five sample days. Letters show significant differences between substrates studied ($p < 0.05$), Tukey-HSD test. (*A*) *Petunia*, (*B*) *Pelargonium*. Huelva, ETSI, 2017.