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Vermicompost enhances germination of the maritime pine (*Pinus pinaster* Ait.)

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Abstract

We investigated the effect of vermicompost on the germination and early development of six different progenies of the maritime pine (*Pinus pinaster Ait*.). We compared the effects of incorporating solid vermicompost into the potting media to those of vermicompost water extract to asses the extent of not physically-mediated positive effects. The incorporation of vermicompost in the growing media of maritime pine increased germination by 16%, and particularly, addition of vermicompost water extract produced the best results. Plants germinated with vermicompost showed higher N content as compared to control plants, and this could have determined the faster maturation of the treated seedlings. Since the best effects on pine germination were observed after application of vermicompost water extract, other mechanisms, rather than the physical amelioration of the substrate, such as the presence of water soluble nutrients and organic compounds (i.e. humic acids and plant growth regulating substances) in the vermicompost, might be involved in the promotion of germination. Variation in the response of the different pine progenies to vermicompost application was observed thereby confirming the necessity of taking into account genetic variability in order to study the potential of vermicompost and other biologically-active organic materials as a potting amendments.

Keywords

Forest nursery; Seed germination; Plant genotype; Genotype x environment interaction; Earthworm compost; Organic amendment.

Introduction

The maritime pine (*Pinus pinaster* Ait.) is the most important forest tree species in south-western Europe. It occupies nearly 1.3, 1.5 and 1 million ha in France, Spain and Portugal, respectively, with a total of 4 million ha in the world including other countries like New Zealand, Australia, Chile, and South Africa. Only in Galicia (NW Spain), maritime pine plantations comprise 400,000 ha (27% of the Galician wooded area) with an annual volume increment estimated around $3 \cdot 10^6$ $m^3 \cdot year^{-1}$ (Xunta de Galicia 2001). Nowadays, *P. pinaster* is the most commonly planted forest tree in this area, with around 3000 – 6000 ha planted annually. Galician forest nurseries need to produce between 4 and 10 million plants yearly to satisfy this high annual planting rate (Zas and Merlo 2008).

Potting media in forest nurseries are normally constituted by peat, bark, perlite and vermiculite, either alone or mixed in different proportions. These materials are used because of their physical properties since they provide adequate aeration, moisture retention and support for the seedlings. Since none of these materials provide relevant nutrient supply to the plant, fertilization is normally accomplished separately either through the incorporation of solid mineral fertilizers into the growing media or, most commonly, through fertirrigation, where mineral fertilizers are dissolved in the irrigation water and supplied regularly to the plant. Nevertheless, fertirrigation generally involves the generation of large amounts of leachates which can easily contaminate soil and groundwater (McAvoy et al. 1992). In addition, the use of peat involves the exploitation of non-renewable resources and the degradation of highly valuable ecosystems like peatlands (Barkham 1993; Robertson 1993). In many countries several restrictions have been established for the use of this material due to environmental concerns and, as a consequence, peat has become a rather scarce and expensive potting substrate. Therefore, in order to reduce costs and adopt more environmentally-friendly practices, research on alternative substrates is of great interest in the future, and several alternatives have already been proposed.

The parallel increasing concern in waste recycling has lead to the proposal of some organic materials such as compost-like substrates (Ostos el al. 2008), as partial substitutes of peat. Sewage sludge compost showed to be an adequate potting amendment for the production of *Pinus pinea* (Guerrero et al. 2002), and Mañas et al. (2009) observed that sewage sludge, composted municipal solid waste and activated sludge could be successfully added to the potting media of maritime pine seedlings with significant improvements in seedling emergence and quality. Compost-like materials normally have a higher nutrient content and ion exchange capacity which allows, not only the reduction of the use of mineral fertilizers, but also the increase of fertirrigation efficiency due to a higher retention of the nutrients in the potting media (Tomati et al. 1990). Therefore, the incorporation of organic fertilizers to the potting media in forest nursery entails both environmental and economic benefits. Among the compost-like materials, vermicompost is gaining interest. In contrast to compost, vermicompost is produced under mesophilic conditions by the joint interaction of earthworms and microorganisms in the organic matter breakdown (Domínguez 2004). Vermicompost can be defined as a humified, stabilized, finely divided peat-like material with a low C: N ratio, high porosity, high water retention capacity and with most nutrients in forms readily available for plants, all these properties resulting from the intense processing and mineralization of a waste carried out by the earthworms during vermicomposting (Domínguez 2004). However, the chemical and biological properties of vermicompost can vary greatly depending on the initial organic waste, the production process, and even on the earthworm species used (Campitelli and Ceppi 2008; Lores et al. 2006). The benefits of the use of vermicomposts as organic amendments in agriculture, ranging from their physical to their biological properties include a slow release source of nutrients that supply the plants with the nutrients when they are needed (Chaoui et al. 2003), improvement of soil and potting substrate physical properties (Kahsnitz 1992; Hidalgo et al. 2006) and microbial activity (Domínguez 2004).

Furthermore, biologically active metabolites such as plant growth regulators (Tomati et al. 1987; El Harti et al. 2001) and humates (Muscolo et al. 1999; Atiyeh et al. 2002; Canellas et al. 2002) have been reported in vermicomposted materials. As a consequence, several studies have shown that various plant species performed better when cultivated with vermicompost at a relatively low proportion (10-20%) of the growing media, than the equivalent mineral fertilization in greenhouse or field trials (Atiyeh et al. 2000; Arancon et al. 2005). Recent studies demonstrated that aqueous extracts of compost and vermicompost can enhance growth and suppress plant disease (Scheuerell and Mahaffee 2004, Arancon et al. 2007). Compost and vermicompost water extracts contain a series of bioactive soluble molecules (Spaccini et al. 2008; Puglisi et al. 2008) as well as microbial populations inhabiting the original compost which might be enhanced during the production of compost extracts depending on the production process. Although there is still insufficient information on the chemical and biological properties of vermicompost extracts, Arancon et al. (2007), and Edwards et al. (2007), demonstrated that the addition of a vermicompost extract to the growing media of tomatoes and cucumbers enhanced germination and growth of these plants. Therefore the use of these extracts might be considered too as an environmentally friendly alternative to water-soluble chemical fertilizers which might also enhance plant health in nurseries.

The effects of vermicompost on plant growth have been studied mainly in horticultural and ornamental plant species while there are few data concerning the effects of vermicompost on the germination and early growth of woody forestry species, and specifically the effects on maritime pine have not been investigated until now. Alves and Passoni (1997) observed that vermicompost increased the germination index of the Brazilian tree species *Licania tomentosa*. Donald and Visser (1989) found that vermicompost had contrasting effects in the growth of the nursery species *Acacia mearnsii*, *Pinus patula* and *Eucalyptus grandis*, which shows that the effect of vermicompost might depend greatly on the species studied.

Furthermore, these effects can vary even among varieties of the same species, as showed by Zaller (2007) in a study with three tomato varieties, evidencing that plant genotype might play a key role in the response to organic fertilizers and more specifically to vermicompost.

Given the large production and the economic importance of the forest nursery sector, research into alternative fertilizers and potting substrates to reduce the use of peat and chemical fertilizers in nurseries is of great importance. Although several studies have shown the feasibility of replacing peat by organic amendments with improvements in seedling quality (Mañas et al. 2009), detrimental effects on plant survival and growth have also been observed due to a worsening of the physical properties of the potting substrate or to the presence of phytotoxical substances (Veijalainen et al. 2007). Thus, even though beneficial effects could be predicted due to the nutrient input, the effects of the organic amendments cannot be generalized or predicted and specific investigations should be carried out both for the different types of organic amendments and plant species.

With the aim to explore the viability of vermicompost as an alternative amendment in forest nurseries, we evaluated in detail the effects of vermicompost in the germination and early ontogenic development of the maritime pine, the first stages of nursery breeding processes. We hypothesized that vermicompost addition might influence the germination and early growth of maritime pine through biological mechanisms as compared to the conventionally used germination substrates in forest nurseries, resulting in a better germination and growth in the seedlings receiving vermicompost. In addition we hypothesized that this effect will depend on plant genotype. The possible physical effects of vermicompost addition were studied through the comparison of the effects of solid vermicompost and vermicompost water extract on seed germination. The role of plant genotype in the response to vermicompost was also evaluated through the assessment of the effects on six different pine progenies.

Material and Methods

Plant material

Pine seeds were obtained from six open pollinated *P. pinaster* clones (A, B, C, D, E, and F) randomly selected from a first generation clonal seed orchard (Sergude, 42.82º N, 8.45º W). This seed orchard provides seeds of high genetic quality for reforestation in the Atlantic region of Galicia (NW Spain).

We interrupted the dormancy of the pine seeds according to commercial procedures by introducing the pine seeds in a water bath with aeration at 24ºC for 24 hours; afterwards seeds were allowed to dry at room temperature and weighed in a precision balance.

Experimental design

We performed a randomized bifactorial experiment including the factors Progeny (six levels) and Vermicompost Treatment (three levels: control, solid vermicompost and vermicompost extract). Potting media consisted on 500 mL plastic pots (Ø 12 cm, height 6.5 cm) filled with perlite with a layer of sand (3 cm) on top, where the following treatments were applied: (i) Control, potting media without treatment addition; (ii) Vermicompost mixed with sand (1:1 v/v) in the top layer of each pot, resulting in 10.75 g of vermicompost per pot; and (iii) Vermicompost water extract (1:15 w:v). Each combination of the experimental factors (pine progeny and vermicompost treatment) was applied to one pot with four replicates resulting in a total of 72 pots (6 progenies x 3 vermicompost treatments x 4 replicates), 12 pots corresponding to each progeny and 24 pots corresponding to each vermicompost treatment.

Eight seeds were sown in each pot, at a depth of one centimeter from the surface, with a distance of 3 cm among them. Pots were randomized and placed into a culture chamber with 12 h light (radiation: 459 µmol m^{-2} s^{-1}) and 12 h darkness at 24 ºC. In order to avoid undesired spatial autocorrelation, the position of the pots in the growth chamber was changed randomly

every day. All pots were watered to field capacity in order to counterbalance the amount of water provided to the plant with the vermicompost extract.

The sand layer in the control and vermicompost water extract treatments had a bulk density of 1.26 g cm^{-3}, pore volume of 45% and water holding capacity of 28% (w w^{-1}). The sand-vermicompost mixture had a bulk density of 0.84 g cm^{-3}, pore volume of 58% and water holding capacity of 66% (w w^{-1}).

Vermicompost and vermicompost extract preparation

Vermicompost was produced by the earthworm *Eisenia fetida* from rabbit manure in 1 m^3 vermirreactors at the facilities of the vermicomposting company Todoverde (Ourense, Spain). Rabbit manure, consisting of a mixture of faeces and urine, was collected in a rabbit farm; it was characterized by 69% organic matter content, electrical conductivity of 0.27 mS cm^{-1}, pH of 7.75, total nitrogen content of 1.92%, and C: N ratio of 15. Ammonia and nitrate contents were 434 ± 228 and 163.95 ± 15.86 µg g^{-1} dw respectively (mean ± s.e). Solid vermicompost had a neutral pH and high contents of dissolved organic carbon and nitrogen (Table 1).

For the vermicompost extracts preparation, the same amount of vermicompost assayed in the solid state (258 g f.w.) was divided into four portions (64.5 g each) which were stored at 4ºC until used. Each week, during the first four weeks of the experiment, one of the portions was diluted into a volume of distilled water equivalent to the sum of the field capacity of the pots treated with the extracts (1080 ml). The solution was aerated for 24 hours using an air pump, filtered through a 0.05 mm sieve and subsequently poured onto the pots surface to field capacity (45 ml per pot). Extracts had similar pH and slightly higher electrical conductivity. Nevertheless, nutrient contents were fairly lower in the extracts than in the solid vermicompost; organic and inorganic nitrogen concentrations were 3 to 4 times lower while the dissolved organic carbon was 20 times lower than in the solid vermicompost (Table 1).

Evaluation of seed germination, seedling ontogenic development and growth

All pots were monitored daily and the date of germination recorded for each seed. The time until the germination of the first seed in each pot (t1, days) and the recruiting time (days for germination of the last seed minus days for germination of the first seed) were calculated. Percentage of final emergence was also calculated for each pot (% germination).

For the assessment of the maturation speed of the seedlings, four early developmental stages were delimited: 1st: emergence of the stem, 2nd: straightening of the stem to an angle of more than 90º; 3rd: appearance of the cotyledons; 4th: appearance of the first set of juvenile needles. Seedling development was monitored daily for 97 days and maturation speed was evaluated as the slope of linear relationship between the ontogenetic stages and the days necessary to reach them for each pine seedling.

Two days after reaching the 4th developmental stage, the seedlings were harvested. Shoot length was measured from the root collar to the base of the cotyledons. Similarly, root length was measured from the root collar to the tip of the radicle. Seedlings were subsequently oven-dried at 60ºC for 48 hours and then weighed for their biomass.

Physical and chemical analysis

The vermicompost was sieved (2 mm) and moisture and organic matter content were calculated gravimetrically after drying at 60ºC for 24 h and ashing at 450ºC for 6 h respectively. The pH and electrical conductivity (EC) of the vermicompost extracts were determined directly, and the pH and EC of the solid vermicompost was determined in water diluted samples (1:20). Inorganic nitrogen (NH_4^+ and NO_3^-) was determined in 0.5 MK_2SO_4 extracts (1:10 w/v) for the solid vermicompost and in the water extracts by the modified indophenol blue technique (Sims et al. 1995), with a microplate reader (Bio-Rad Model 550). Total extractable N was determined after oxidation with $K_2S_2O_8$ as described by Cabrera and

Beare (1993) and the dissolved organic nitrogen (DON) content was calculated as (total extractable N)–(NH_4^+ –N +NO_3^- –N) both in the K_2SO_4 and water extracts. Dissolved organic carbon (DOC) was determined colorimetrically at 590 nm after moist digestion ($K_2Cr_2O_7$ and H_2SO_4) of aliquots of the water and 0.5 M K_2SO_4 extracts (1:10 w/v). Total N content of the dried and ground seedlings was determined on a Carlo Erba 1500 C/N analyzer.

Bulk density, pore volume and water holding capacity of the sand and vermicompost-sand mixture used as final germination media were determined following standard procedures as outlined in TMECC method 03.01 (Thomson et al. 2003).

Data analysis

For the parameters measured per pot (germination parameters and total N content of the seedlings) a full bifactorial ANOVA was used, with progeny and treatment as main fixed factors. Mean seed weight of the pots was introduced as a covariate in the model. For the parameters measured per seedling (maturation speed and growth), the pot factor was included in the model since pine seedlings sown in the same pot were not independent and the individual seed weight of the seedlings was introduced as a covariate in the model. In all cases significant differences were further analyzed with Fisher LSD test. Parameters distributions were analyzed for normality by Kolmogorov-Smirnov criterion and homogeneity of variances by Levene's test. Transformations using square root were enough to meet analysis of variance (ANOVA) requirements. Data were analyzed using the STATISTICA v7 software program.

Results

Seedling germination

The different treatments assayed influenced significantly the germination percentage of the seedlings independently of the progeny considered (Table 2); the addition of

vermicompost extracts to the germination media produced a 16% increase in the germination percentage at the end of the experiment as compared to the control treatment (Fig. 1a). The six pine progenies studied showed different germination percentage (Tables 2, 4); whereas final germination in progenies A and B was 78 and 76 %, this parameter was significantly lower in progenies D, E and F (52, 41 and 51 % respectively). No effect of the seed weight was observed in seed germination.

The addition of vermicompost, either solid or liquid, to the germination media did not influence the start of seed germination (t1) (Table 2; Fig. 1b). No significant differences were observed either in this parameter among the different progenies. Nevertheless the start of germination seemed to be strongly influenced by the seed weight, and bigger seeds tended to germinate faster than the smaller ones (Table 2).

Solid vermicompost added into the germination media increased significantly the recruiting time of the *P. pinaster* seeds independently of the progeny considered (Fig. 1c). No differences were observed among the different progenies in their recruiting time, and no effect of seed weight in this parameter was observed either (Tables 2, 4).

Seedling growth

The treatments assayed influenced significantly the biomass of the seedlings independently of the progeny considered (Table 3). Vermicompost application, either solid or as an extract, reduced significantly the biomass of the seedlings as compared to the control media. Variations in biomass between the treatments were mainly due to a significant decrease in root biomass of the pines grown with solid and liquid vermicompost in the germination media (3.7 ± 0.11 and 3.8 ± 0.08 mg respectively) as compared to the control seedlings (4.4 ± 0.12 mg). On the contrary, the treatments had no effect on aerial biomass which was only influenced by the progeny and the seed mass (Tables 3, 4).

In spite of this, shoot length was significantly decreased by vermicompost incorporation, although these effects depended on the progeny (Table 3). Shoot length was decreased by vermicompost incorporation in progenies A, E and F and increased by the vermicompost extract in progeny C, while shoot length in progenies B and D remained unaffected (Fig. 2). Root length was also decreased with vermicompost incorporation, although this depended on the progeny considered (Table 3). Generally root length was significantly reduced by the vermicompost extract; however both solid vermicompost and vermicompost extract reduced root length in progeny B, while this parameter was reduced only by solid vermicompost in progeny A (Table 3, Fig.2). No effect of the seed weight was observed in shoot and root length (Table 3).

Seedling ontogenic development

Vermicompost addition to the germination media produced significant changes in the maturation speed of the seedlings, although the effects of vermicompost varied depending on the progeny (Table 3). Vermicompost, either in the solid state or as a water extract produced a significant increase in the maturation speed of the progenies D and E, and a significant decrease in this parameter in the maturation speed of progeny C. No effects of vermicompost were observed in the maturation of the progenies B and F, while progeny A was affected differently by solid vermicompost and vermicompost extracts (Fig. 3). In addition we observed a strong effect of the seed weight in this parameter (Table 3).

Seedling tissue nitrogen content

There was a clear effect of the treatments on the nitrogen assimilation by the pine seedlings (Table 2). Those grown with vermicompost, either solid or liquid, into the germination media showed significantly higher nitrogen content (86.6±0.05 and 85.5±0.09 mg g^{-1} for vermicompost and vermicompost extract respectively) than the untreated control

(83.2±0.11 mg g^{-1}). These effects were similar for all the progenies. The different pine progenies showed differences in their nitrogen assimilation but no effect of the seed weight was found (Table 2). Generally, progenies A and D showed the highest nitrogen content while progenies E and F showed the lowest content (Table 4).

Discussion

Germination is an internally regulated process influenced mainly by genotype although external factors such as light period, temperature, moisture, and presence of certain chemical compounds, can also alter this process either through promotion or inhibition (Kucera et al. 2005). All this information is integrated and this is mediated by signalling through multiple hormones that either promote, or inhibit germination (Finkelstein 2004).

Germination of the pine seeds showed to be strongly influenced by plant genotype. As expected, there were evident differences among the different progenies in their ability to germinate and grow, although some parameters like recruiting time, shoot and root lengths, were not affected by plant genotype and seemed to be dependent of the environmental conditions more that by genetic traits. Seed weight, accounting for genetic and environmental maternal effects (Castro 1999), conditioned the start of the germination of the pools of seeds (larger seeds germinated faster that the smaller ones), and also influenced significantly the speed of maturation and final biomass of the seedlings.

In addition to the strong effects of the progeny and seed weight (accounting for the genetic sources of variability), pine germination was favoured by the addition of vermicompost with a 16% increase in the number of germinated seeds with vermicompost extracts as compared to the control. Vermicompost has shown to stimulate germination of several ornamental and horticultural plant species such as green gram (*Phaseolus aureus*) (Karmegam et al. 1999) tomato plants (*Lycopersicon esculentum*) (Atiyeh et al. 2000; Zaller 2007) and petunia (Arancon et al. 2008). Similarly the addition of increasing doses of

vermicompost to the potting media of *Licania tomentosa*, a woody forestry species, resulted in a greater germination index and growth of the plants as compared to the unamended soil (Alves and Passoni 1997). Even though the effects of vermicompost on maritime pine germination have not been investigated until now, other studies have shown that germination and growth of this species can be improved after the addition of different sources of organic matter to the potting media (Mañas et al. 2009, but see Ribeiro et al. 1999).

Changes in the physical properties of the germination media after the incorporation of solid vermicompost influenced the moisture retention and aeration of the substrate thereby potentially affecting seed germination. Nevertheless, in our experiment percentage of final germination was increased by vermicompost extract, where the physical effects of vermicompost incorporation were absent and the nutrients supplied were much lower than with the solid vermicompost, thus suggesting that other factors rather than physical amelioration were responsible for higher germination. A recent study by Spaccini et al. (2008) showed that aerated compost extracts contained most of the low-weight compounds associated to a compost matrix, most of them of microbial origin and therefore potentially bioactive substances. Moreover, a further study demonstrated that these extracts produced greater effects in plant physiology than the equivalent bulk compost (Puglisi et al. 2008). The presence of bioactive substances associated to the low molecular weight fraction of the humic acids, capable of inducing changes in plant morphology and physiology has also been reported in vermicompost (Canellas et al. 2002; Quaggiotti et al. 2004). Therefore, water-soluble bioactive substances, such as humic acids, water soluble PGRs, or microorganisms present in vermicompost extract could have been responsible for the increased germination of the pine seeds.

In addition to the observed increases in seed germination, vermicompost application accelerated the maturation of three out of the six progenies studied. Furthermore, seedling biomass, which was under strong genetic control, decreased after vermicompost addition

(either solid or liquid) due to the decrease of root biomass regarding to the control. The observed changes in seedling maturation and biomass after addition of vermicompost are probably related to the different nutrient availability in the different growing media. It is possible that the seedlings growing in the control media enlarged their root biomass to compensate for the lower nutrient availability as explained by the optimal partitioning theory (McCarthy and Enquist 2007). On the contrary, the investment in roots, high maintenance respiration tissues, was not necessary in the seedlings grown in the vermicompost derived products as showed by their higher N tissue content. Decreases in root biomass with increasing nutrient availability were also observed by Albaugh et al. (1998) and Müller et al. (2000) in Loblolly pine and several herbaceous species respectively.

In spite that sand and perlite is a rather poor medium to be compared to vermicompost, this type of medium is being extensively used in forest nurseries as hydroponic germination medium and no other special care or additives but water are supplied during germination since they are not necessary. The introduction of an organic material such as vermicompost has never been considered before and, it can bring clear and significant improvements on seed germination. The results observed in this experiment demonstrate that the use of vermicompost allows a significant increase in the productivity and efficiency of the forest nurseries, due to the increase in the germinated seeds and to a faster maturation of the seedlings. Even though the results showed here correspond to a very early developmental stage, where seedling quality cannot still be foreseen, the higher germination rate and development will condition further seedling growth within the nursery, thereby conditioning also plant vigour in latter stages and post-transplant success. The consequences of the decreased plant biomass and increased tissue N contents for post transplant growth and survival still need to be investigated and will depend greatly on the environmental conditions after transplanting (Jacobs et al. 2003; Mañas et al. 2009). For instance, the decrease in root biomass of the seedlings could result detrimental for the survival of newly-planted seedlings

in the field since root morphology determines the amount of soil that can be exploited by the plants and will therefore influence the uptake of nutrients and water (Benedikz et al. 2005). The high nitrogen content indicates a better nutritional status of the seedlings and would determine a greater vigour than the control plants thereby ensuring plant health and further growth under field conditions in spite of the reduced development of the root system in the nursery containers.

Although most of the effects of vermicompost were constant among the progenies, for some parameters like maturation speed and shoot and root length intraespecific variation in the response of the seedlings to vermicompost application was observed thereby confirming the necessity of taking into account genetic variability in order to study the potential of vermicompost and other biologically-active organic materials as a potting amendments.

Conclusion

Vermicompost seemed to be an adequate amendment for pine seed germination increasing the number of seeds germinated and accelerating seedling development. Vermicompost incorporation into the germination media produced seedlings with lower root biomass but higher nutrient content than the control. The higher nutrient availability after vermicompost addition seems to be responsible for decreased growth and faster maturation of the seedlings but other mechanisms such as the presence of biologically active substances in vermicompost that might be involved in the promotion of germination still need to be investigated.

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References

Albaugh TJ, Allen HL, Dougherthy PM, Kress LW, King JS (1998) Leaf area and above- and belowground responses of loblolly pine to nutrient and water additions. For Sci 44: 317-328.

Alves WL, Passoni AA (1997) Compost and vermicompost of urban solid waste in *Licania tomentosa* (Benth) seedlings production to arborization. Pesqu Agropec Bras 32: 1053-1058.

Arancon NQ, Edwards CA, Babenko A, Cannon J, Galvis P, Metzger JD (2008) Influences of vermicomposts, produced by earthworms and microorganisms from cattle manure, food waste and paper waste, on the germination, growth and flowering of petunias in the greenhouse. Appl Soil Ecol 39: 91-99.

Arancon NQ, Edwards CA, Bierman P, Metzger JD, Lucht C (2005) Effects of vermicomposts produced from cattle manure, food waste and paper waste on the growth and yield of peppers in the field. Pedobiologia 49: 297-306.

Arancon NQ, Edwards CA, Dick R. Dick L (2007) Vermicompost tea production and plant growth impacts. Biocycle 48 (11): 51-52.

Atiyeh RM, Arancon NQ, Edwards CA, Metzger JD (2000) Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes. Bioresource Technol 75: 175-180.

Atiyeh RM, Arancon NQ, Edwards CA, Metzger J-D (2002) The influence of earthworm-processed pig manure on the growth and productivity of marigolds. Bioresource Technol 81: 103-108.

Barkham JP (1993) For peat's sake: conversation or exploitation? Biodivers Conserv 2: 255–566.

Benedikz T, Ferrini F, Garcia-Valdecantos JL,·Tello ML (2005) Plant quality and establishment. In: Konijnendijk et al. (eds) Urban forests and trees. Part III. Springer Berlin Heidelberg, pp 231-256.

Cabrera ML, Beare MH (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci Soc Am J 57: 1007-1012.

Campitelli P, Ceppi S (2008) Effects of composting technologies on the chemical and physicochemical properties of humic acids. Geoderma 144: 325-333.

Canellas LP, Olivares FL, Okorokova-Façanha AL, Façanha AR (2002) Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H+-ATPase activity in maize roots. Plant Physiol 130: 1951-1957.

Castro J (1999) Seed mass versus seedling performance in Scots pine: a maternally dependent trait. New Phytol 144: 153–161

Chaoui HI, Zibilske LM, Ohno T (2003) Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. Soil Biol Biochem 35: 295-302.

Domínguez J (2004) State of the Art and New Perspectives on Vermicomposting Research. In: Edwards CA (ed) Earthworm Ecology, 2nd ed. CRC Press, Boca Raton, FL, USA, pp, 401-424.

Donald DGM, Visser LB (1989) Vermicompost as a possible growth medium for the production of commercial forest nursery stock. Appl Plant Sci 3: 110-113.

Edwards CA, Arancon NQ, Emerson E, Pulliam R (2007) Suppressing plant parasitic nematodes and arthropod pests with vermicompost teas. Biocycle 48: 12, 38-39.

El Harti A, Saghi M, Molina J-AE, Téller G (2001) Production d'une substance rhizogène à effet similaire à celui de l'acide indole acètique par le ver de terre *Lumbricus terrestris*. Can J Zool 79: 1911-1920.

Finkelstein RR (2004) Hormones in seed development and germination. In: Davies PJ (ed) Plant hormones: biosynthesis, signal transduction and action! Kluwer Academic Publishers, pp 513-537.

Guerrero F, Gascó JM, Hernández-Apaolaza L (2002) Use of pine bark and sewage sludge composts as components of substrates for *Pinus pinea* and *Cupressus arizonica* Production. Compos Sci Util 25:129-141.

Hidalgo PR, Matta FB, Harkess RL (2006) Physical and chemical properties of substrates containing earthworm castings and effects on marigold growth. HortScience 41: 1474-1476.

Jacobs DF., Rose R, Haase DL, Morgan PD (2003) Influence of nursery soil amendments on water relations, root architectural development, and field performance of Douglas-fir transplants. New Forest 26: 263-277.

Kahsnitz HG (1992) Investigations on the influence of earthworms on soil and plant. Bot Arch 1: 315-351.

Karmegam N, Alagumalai K, Daniel T (1999) Effect of vermicompost on the growth and yield of green gram (*Phaseolus aureus* Roxb.). Trop Agr 76: 143-146.

Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15: 281-307.

Lores M, Gómez-Brandón M, Pérez-Díaz D, Domínguez J (2006) Using FAME profiles for the characterization of animal wastes and vermicomposts. Soil Biol Biochem 38: 2993-2996.

Mañas P, Castro E, de las Heras J (2009) Quality of maritime pine (*Pinus pinaster* Ait.) seedlings using waste materials as nursery growing media. New Forest 37: 295-311.

McAvoy RJ, Brand MH, Corbett EG, Bartok Jr JW, Botacchi A (1992) Effect of leachate fraction on nitrate loading to the soil profile underlying a greenhouse crop. J Environ Hortic 10: 167-171.

McCarthy MC, Enquist BJ (2007) Consistency between an allometric approach and optimal partitioning theory in global patterns of plant biomass allocation. Funct Ecol 21: 713-72.

Müller I, Schmid B, Weiner J (2000) The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. Perspect Plant Ecol Evol Syst 3(2): 115-127.

Muscolo A, Bovalo F, Gionrtiddo F, Nardi S (1999) Earthworm humic matter produces auxin-like effects on *Daucus Carota* cell growth and nitrate metabolism. Soil Biol Biochem 31: 1303-1311.

Ostos JC, López-Garrido R, Murillo JM, López R (2008) Substitution of peat for municipal solid waste- and sewage sludge-based composts in nursery growing media: effects on growth and nutrition of the native shrub *Pistacia lentiscus* L. Bioresource Technol 99: 1793-1800.

Puglisi E, Fragoulis G, Del Re AAM, Spaccini R, Piccolo A, Gigliotti G, Said-Pullicino D, Trevisan M (2008) Carbon deposition in soil rhizosphere following amendments with compost and its soluble fractions, as evaluated by combined soil-plant rhizobox and reporter gene systems. Chemosphere 73: 1292-1299.

Quaggiotti S, Ruperti B, Pizzeghello D, Francioso O, Tugnoli V, Nardi S (2004) Effect of low molecular size humic substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.). J Exp Bot 55:803-813.

Ribeiro H, Ribeiro D, Vasconcelos E, Cabral F, Louro V, dos Santos JQ (1999) Evaluation of a sewage sludge based compost for the production of container tree seedlings. In: Anac D, Martin-Prével P (eds). Improved crop quality by nutrient management. Springer Netherlands, pp 181-184.

Robertson RA (1993) Peat, horticulture and environment. Biodivers Conserv 2: 541–547.

Scheuerell SJ, Mahaffee WF (2004) Compost Tea as a Container Medium Drench for Suppressing Seedling Damping-Off Caused by *Pythium ultimum*. Phytopathology 94: 1156- 1163.

Sims GK, Ellsworth TR, Mulvaney RL (1995) Microscale determination of inorganic nitrogen in water and soil extracts. Commun Soil Sci Plant 26: 303–316.

Spaccini R, Baiano S, Giliotti G, Piccolo A (2008) Molecular characterization of a compost and its water-soluble fractions. J Agr Food Chem 56: 1017-1024.

Thompson W, Leege P, Millner, P, Watson ME (2003). Test methods for the examination of composts and composting. The US composting council (ed) US Government Printing Office http://tmecc.org/tmecc/index.html.

Tomati U, Galli E, Grappelli A, Dihena G (1990) Effect of earthworm casts on protein synthesis in radish (*Raphanus sativum*) and lettuce (*Lactuca sativa*) seedlings. Biol Fertil Soil 9: 288–289.

Tomati U, Grappelli A, Galli E (1987) The presence of growth regulators in earthworm-worked wastes. In: Bonvicini Paglioi AM, Omodeo P (eds) On earthworms. Modena, Italy, pp 423-435.

Veijalainen AM, Juntunen ML, Heiskanen (2007) Growing *Picea abies* container seedlings in peat and composted forest-nursery waste mixtures for forest regeneration. Scand J Forest Res 22: 390-397.

Xunta-de-Galicia (2001) O monte galego en cifras. Xunta de Galicia, Dirección Xeral de Montes e Medio Ambiente Natural. Santiago de Compostela, Spain, p 227.

Zaller JG (2007) Vermicompost as a substitute for peat in potting media: Effects on germination, biomass allocation, yields and fruit quality of three tomato varieties. Sci Hortic –Amsterdam 112: 191-199.

Zas R, Merlo E (2008) El programa de mejora de *Pinus pinaster* en Galicia. Boletin CIDEU. In press. Available online at www.uhu.es/cideu/

Figure legends

Figure 1. Percentage of germination (a), time for the germination of the first seed (t1) (b), and recruiting time (c) of the pine seedlings germinated in sand (control), and sand amended with vermicompost and vermicompost extract. Values are overall means ± standard error of all the pine progenies assayed. Different letters indicate significant differences at $p < 0.05$ (Fisher LSD test).

Figure 2. Root and shoot length of the different progenies studied (A, B, C, D, E and F) germinated in sand (control), and sand amended with vermicompost (VC) and vermicompost extract, at the second day of the fourth developmental stage. Values are means ± standard error. Different letters indicate significant differences at $p < 0.05$ (Fisher LSD test).

Figure 3. Maturation speed of the different progenies studied (A, B, C, D, E and F) germinated in sand (control), and sand amended with vermicompost (VC) and vermicompost extract. Values are means ± standard error. Different letters indicate significant differences at $p < 0.05$ (Fisher LSD test).

Table 1. Physicochemical characteristics of the vermicompost and vermicompost extracts used in this experiment. Values are means of three replicates.

	Vermicompost	Vermicompost extract
pH	7.32	7.74
EC (mS cm^{-2})	0.29	0.38
DOC	4967 μg g dw $^{-1}$	246 μg mL^{-1}
DON	2241 μg g dw $^{-1}$	586 μg mL^{-1}
$N\text{-}NH_4^+$	15 μg g dw $^{-1}$	4.6 μg mL^{-1}
$N\text{-}NO_3^-$	1303 μg g dw $^{-1}$	333 μg mL^{-1}

EC: electrical conductivity; DOC: dissolved organic carbon; DON: dissolved organic nitrogen; dw: dry weight.

Table 2. ANOVA results of the effect of the family, the treatment with vermicompost amendments and seed weight (covariate) on the germination, time to first emergence (t1), recruiting time, and total N concentration in the pine seedlings.

	Progeny		Treatment		Progeny x Treatment		Seed weight	
	F	p	F	p	F	p	F	p
% Germination	6.54	< 0.01	5.12	0.01	1.03	0.43	1.19	0.28
t1	1.88	0.11	1.46	0.24	0.85	0.57	6.91	0.01
Recruiting time	1.72	0.18	6.87	< 0.01	0.37	0.95	3.72	0.06
Total tissue N	9.14	< 0.01	7.33	< 0.05	1.59	0.13	1.50	0.22

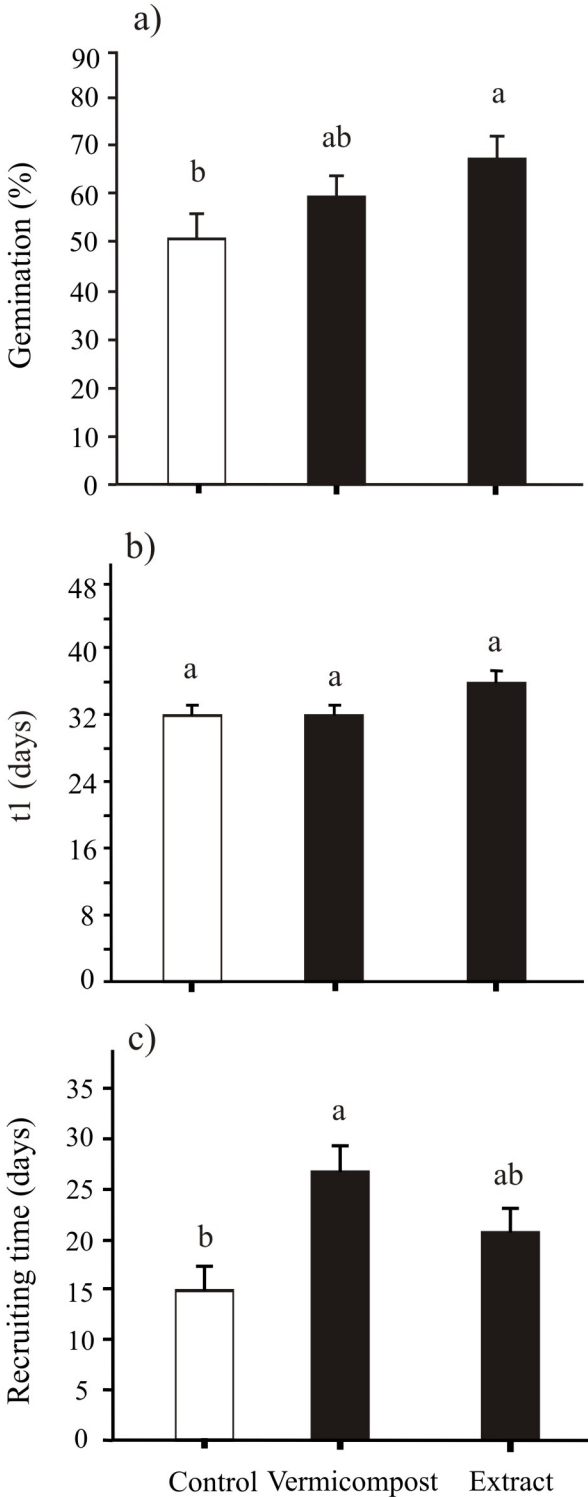
Table 3. ANOVA results of the effect of the family, the treatment with vermicompost amendments and seed weight (covariate) on the ontogenic development (maturation speed) and growth parameters (shoot and root biomass, shoot and root length) measured in the pine seedlings.

	Family		Treatment		Family x Treatment		Pot		Seed weight	
	F	p	F	p	F	p	F	p	F	p
Maturation speed	4.70	< 0.01	9.05	< 0.01	2.50	< 0.01	2.19	< 0.01	20.27	< 0.01
Seedling total biomass	70.21	< 0.01	4.91	< 0.01	1.194	0.31	1.51	0.01	548.26	< 0.01
Shoot biomass	67.72	< 0.01	2.86	0.05	1.49	0.14	1.55	0.01	588.95	< 0.01
Root biomass	35.57	< 0.01	6.64	< 0.01	1.21	0.28	1.43	0.03	59.24	< 0.01
Shoot length	1.57	0.16	4.64	0.01	2.54	< 0.01	1.64	< 0.01	0.09	0.76
Root length	1.03	0.40	17.16	< 0.01	4.10	< 0.01	1.58	0.01	0.30	0.58

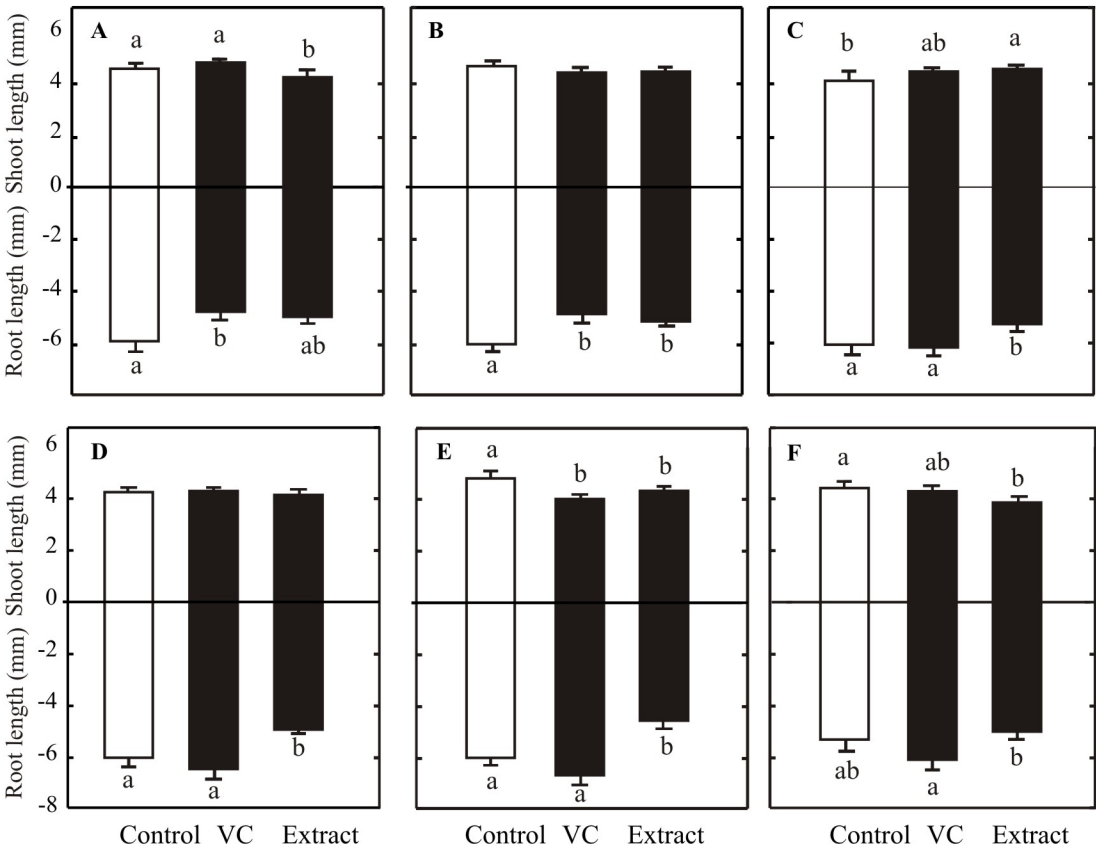
Table 4. Germination and growth parameters of the different progenies of pine seedlings studied. Values are means of the three treatments assayed ± standard errors and different letters in each row indicate significant differences at $p < 0.05$ (Fisher LSD test).

	A	B	C	D	E	F
% Germination	78.1 ± 6.17^{a}	76.1 ± 4.20^{a}	55.2 ± 6.04 ^{ab}	52.1 ± 6.31 ^{ab}	41.6 ± 4.94 ^{b}	51.0 ± 4.29 ^{ab}
t1 (d)	31.8 ± 1.70 ^{ab}	32.8 ± 1.02 ^{ab}	31.7 ± 0.94 ^{ab}	36.3 ± 3.17 ^{ab}	27.4 ± 3.71 ^{a}	38.7 ± 2.36 ^{b}
Recruiting time (d)	22.1 ± 3.69	25.5 ± 3.92	13.9 ± 3.66	29.1 ± 4.20	21.6 ± 3.87	15.9 ± 3.56
Shoot biomass (mg)	23.3 ± 0.00 ^{a}	19.8 ± 0.00 ^{c}	21.9 ± 0.00 ^{b}	17.4 ± 0.00 ^{d}	19.3 ± 0.00 ^{c}	13.5 ± 0.00 ^{e}
Root biomass (mg)	4.7 ± 0.00^{b}	3.7 ± 0.00^{d}	5.1 ± 0.00^{a}	3.4 ± 0.00^{e}	3.6 ± 0.00^{de}	2.9 ± 0.00^{c}
Total N in plant tissue mg g^{-1}	89.1 ± 0.03^{a}	84.6 ± 0.09^{cd}	85.4 ± 0.08^{bc}	88.3 ± 0.10^{ab}	81.5 ± 0.13^{d}	81.5 ± 0.12^{d}

Lazcano et al.Figure 1



Lazcano et al. Figure 2



Lazcano et al. Figure 3