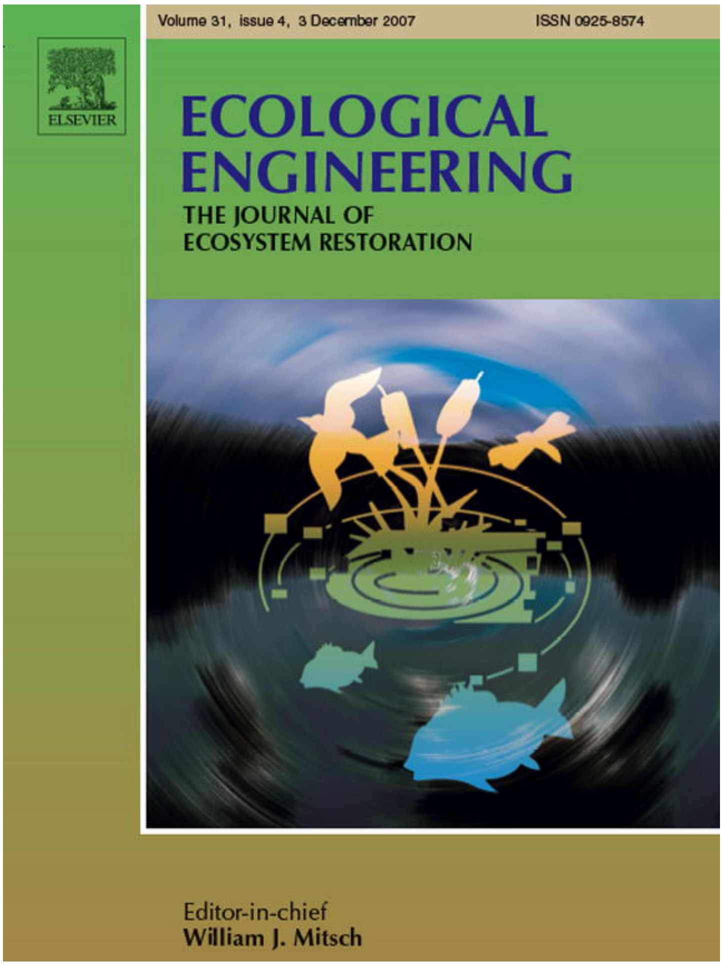
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Seedling performance in sewage sludge-amended degraded mediterranean woodlands

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ABSTRACT

Biosolids have been widely used for land reclamation, but information on their use in restoration, i.e., on less degraded areas, is scarce. Biosolids may be used to restore forest ecosystems by fostering tree establishment in degraded shrublands. Detailed knowledge on the effects of biosolid application is needed to optimize such practice. We evaluated the effect of different rates (0, 7.5 and 14.5 kg dry weight per plant) and types of biosolid application on the performance of *Pinus halepensis* and *Quercus ilex* seedlings, and operational costs. Biosolids increased seedling mortality in both species, particularly when seedlings were planted in direct contact with them. Mortality mostly occurred during the first year, and was probably favored by soil shrinking and salinity. Foliar and needle nitrogen concentration increased with biosolid rate in the short term, but biosolids affected negatively (*P. halepensis*), or had no effect (*Q. ilex*) on phosphorus and potassium concentration. Biosolids had a positive effect on *P. halepensis* growth, and a negative effect on *Q. ilex* growth at the highest rate when seedlings were in contact with biosolids. Cost of this type of biosolid application approximately doubled plantation cost, but were similar or cheaper that landfill disposal of biosolids. The lowest application rate showed the best balance between seedling response and costs for *P. halepensis*, whereas biosolid use cannot be recommended for *Q. ilex*.

1. Introduction

Forest soils in the Mediterranean basin have been intensively exploited for centuries (Naveh, 1982), resulting in a loss of soil organic matter and nutrients (Martínez-Mena et al., 2002). Under these conditions soils often show evidence of nutrient limitation, particularly for phosphorus and nitrogen (Rodà et al., 1999; Sardans et al., 2006), and this may hamper vegetation recovery in degraded soils (Vallejo et al., 2000). Biosolids represent an easily accessible source of organic matter and nutrients and can be used to restore degraded ecosystems. Biosolid production has dramatically increased in the last decade due to more rigorous European directives (Commission of the European Communities, 1991) on wastewater quality. Sewage sludge reutilization has been recommended as the best practicable environmental option for the management of this organic residue (Hall, 1999). Agricultural activities currently absorb half of the biosolids produced in water treatment plants (USEPA, 1999; European Commission, 2000), but environmental risks may impose restrictions on its further

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use (European Commission, 2000). Biosolid applications for the reclamation of degraded areas (Sort and Alcañiz, 1996; Cogliastro et al., 2001; Wilden et al., 2001) and for the production of growth substrates (Hernández-Apaolaza et al., 2005) represent novel scenarios for reutilization. Detailed information on the effects of biosolids on ecosystems is needed to maximize the benefits and minimize the environmental risks of biosolid use in ecological restoration.

Recently, several studies have evaluated the effect of the application of biosolids and other organic amendments on the performance of planted Mediterranean species (Sort and Alcañiz, 1996; Caravaca et al., 2003; Alguacil et al., 2004; Larchevêque et al., 2006; Fuentes et al., 2007). Most of these studies have focussed on the restoration of heavily degraded areas such as old-fields and mines. A quite different case is the restoration of degraded shrublands, which may partly retain the components and functions of an undamaged ecosystem (Cortina et al., 2006). In this latter case, restoration actions aim at establishing key components of the target ecosystem while preserving, and eventually taking advantage of, the remaining ecosystem functions (Harris and van Diggelen, 2006). Moreover, localized biosolid application (i.e., in the planting holes) may be a better option here than widespread biosolid application and soil incorporation. The application of high but spatially localized doses of biosolids may help to minimize the surface area affected by plantation practices, thus minimizing the damages to the surrounding natural vegetation and reducing the risk of off-site contamination (Loch et al., 1995). Moreover, biosolid application at the planting hole prior to planting may help to reduce costs (Valdecantos et al., 2001) and improve social acceptance by avoiding offensive odors and insect proliferation. At present, scarce information is available on the application rates and types that could optimize the use of biosolids in the restoration of degraded Mediterranean shrublands. This is particularly true at the management scale, as few studies have taken into account the ecological, logistic and economic aspects of large-scale biosolid application.

Pinus halepensis and *Quercus ilex* are two common western Mediterranean tree species that differ in their morphological and physiological traits (Pausas et al., 2004). They have been considered as early-successional and shade intolerant species the former and late-successional and shade tolerant the latter (Broncano et al., 1998). For sustainable reforestation actions in Mediterranean landscapes, pines and broad-leaved resprouting species (especially oaks) should be combined to take advantage of the complementary features of both species groups, i.e., the faster growth of pines and the high post-disturbance resilience of oaks (Pausas et al., 2004). Seedlings of Aleppo pine show better performance in a wider range of environmental conditions than Holm oak (Broncano et al., 1998), but oaks' resprouting capacity provides higher resilience during the critical first years after planting. The implementation of pre and post-planting treatments in the artificial introduction of these species in degraded Mediterranean areas show different responses in survival and/or growth (Díaz and Roldán, 2000; Bocio et al., 2004; Navarro Cerrillo et al., 2005; Larchevêque et al., 2006).

Our hypothesis is that a localized application of this type of biosolid can improve seedling performance of two forest tree species (*P. halepensis* and *Q. ilex*) planted on a degraded Mediterranean shrubland in an economically feasible way, as compared with other sewage sludge disposal routes. The aim of the present work is to evaluate the effect of biosolids in seedling survival and growth, especially focusing on the first summer after planting which is the period where most mortality is recorded under Mediterranean dry and semiarid conditions. Nutrient concentration in above-ground tissues, water status, biomass allocation and soil properties were considered to assess the ecological effects of this type of biosolid application on the target seedlings.

2. Materials and methods

2.1. Site

The study area is located in Zarra (Valencia, E Spain) at an altitude of 750 m above sea level (39°4′ latitude, 1°7′ longitude). Climate is dry-subhumid Mesomediterranean (406 mm and 12 °C: mean annual rainfall and temperature, respectively). The soil is a highly carbonated sandy-loam *Cambisol calcaric*, developed from marlstone (Table 1). The site had been heavily grazed for decades, resulting in a low shrubland dominated by *Rosmarinus officinalis*, *Cistus* spp. and *Ulex parviflorus*, with isolated adult trees of *P. halepensis* and *Q. ilex* and a low plant cover.

Table 1 – Physicochemical composition of the soil (mean ± standard error of N = 9) in the experimental area and of the sewage sludge used in the study (OM: organic matter; OC: organic carbon; EC: electrical conductivity; C/N: carbon/nitrogen ratio)

	Soil	Sewage sludge
Clay (%)	15 ± 1	–
Lime (%)	23 ± 1	–
Sand (%)	62 ± 1	–
Dry matter (%)	–	21.4
OM[a] (%)	3.4 ± 0.5	54.8
OC[a] (%)	2.0 ± 0.3	29.4
pH (1:2.5)	8.2 ± 0.1	7.4
EC (1:5) ($dS\,m^{-1}$)	0.21 ± 0.01	4.2
Total N (%)	0.17 ± 0.02	2.7
N-NO_3^- ($mg\,kg^{-1}$)	–	68
N-NH_4^+ ($mg\,kg^{-1}$)	42 ± 7	1644
C/N	11.7 ± 0.4	10.7
$CaCO_3$ (%)	53 ± 2	–
P Olsen ($mg\,kg^{-1}$)	8.1 ± 1.6	–
P total (%)	–	4.9
K total (%)	–	0.19
Ca (CaO) (%)	–	11
Mg (%)	–	0.76
Na (%)	–	0.11
Fe ($mg\,kg^{-1}$)	–	30,264
Cu ($mg\,kg^{-1}$)	–	406
Mn ($mg\,kg^{-1}$)	–	158
Zn ($mg\,kg^{-1}$)	–	1036
Ni ($mg\,kg^{-1}$)	–	47
Pb ($mg\,kg^{-1}$)	–	182
Cd ($mg\,kg^{-1}$)	–	3.3
Cr ($mg\,kg^{-1}$)	–	267
Hg ($mg\,kg^{-1}$)	–	1.8

[a] Oxidable fractions; blank spaces: not determined.

Table 2 – Characteristics of the planting hole and biosolid distribution depending on the dose and type of biosolid application

Treatments[a]	Length (m)	Width (m)	Surface (m^2)	Biosolid layer depth (cm)[b]	Biosolid layer thickness (cm)[c]
H0	0.75 ± 0.06 bc	0.53 ± 0.04 bc	0.39 ± 0.07 bc	–	–
H7	0.69 ± 0.04 c	0.50 ± 0.02 c	0.35 ± 0.03 c	8.4 ± 1.0 a	10.0 ± 0.8 a
H14	0.82 ± 0.04 ab	0.56 ± 0.01 ab	0.46 ± 0.02 b	12.8 ± 2.1 a	14.9 ± 1.4 b
L14	1.10 ± 0.06 a	0.60 ± 0.01 a	0.66 ± 0.04 a	9.2 ± 1.2 a	9.3 ± 0.5 a

Mean and standard error of $N = 9$ planting holes are shown for each treatment. Results of Tukey's test ($P < 0.05$) are indicated by letters within the same column.

[a] H0: control, H7: 7.5 kg d.w. planting hole^{-1}, H14: 14.5 kg d.w. planting hole^{-1}, L14: 14.5 kg d.w. planting hole^{-1} (enlarged holes).

[b] Vertically, from soil surface to sludge patches.

[c] Soil-sludge layer.

2.2. *Biosolid application*

In February 2000, a backhoe excavator dug planting holes of two different sizes along 1.46 ha of gentle, south-facing slopes (0–20%). Regular holes (H) had a smaller surface than enlarged ones (L) (Table 2). As the experimental area was a part of a pilot project and heavy machinery was used, small differences in the size of the planting holes were observed. Anaerobically digested biosolids from a domestic wastewater treatment plant (Pinedo I, located at the city of Valencia) were applied and mixed with the soil at three rates: 0, 7.5 and 14.5 kg dry weight (d.w.) per planting hole. In the enlarged holes (L) we only applied the highest dose, resulting in four different application treatments: H0, H7, H14 and L14. As planting density was 820 seedlings per hectare, the total amount of biosolid applied was 0, 6 and 12 Mg d.w. ha^{-1} in H0, H7, and H14 and L14, respectively. Doses were selected to optimize the economic and ecological viability of the application (Valdecantos et al., 2001). We controlled the time employed by the machinery in each operation (biosolid loading, transport and application) to assess economic viability of treatments. Therefore, treatments were patchy distributed along the pilot project whole area. Two different locations of the introduced seedling were assessed in relation to the planting hole and the biosolid applied: in the center (c) or in the periphery (p). In unamended holes, seedlings were only planted in the center of the hole. For each combination of species, biosolid amount and location of the seedling we planted 75 seedlings (totalizing 1200 seedlings) of 1-year-old *P. halepensis* Mill. and *Q. ilex* subsp. *ballota* Desf. grown in a nearby nursery in containers filled with peat and coconut fiber.

2.3. *Soil characterization*

We measured the surface area affected by soil preparation and we did a cross section through the soil of the planting hole to assess the vertical distribution of the biosolids in 9 holes per treatment, randomly selected, regardless of seedling location (Table 2). Soil salinity (0–30 cm depth) was monitored both in the unamended planting holes and in those receiving the highest dose (H14), by measuring the electrical conductivity (EC) of 1:5 (w/v) solution of soil and deionized water on four different dates during the first 2 years after planting. It was also measured in all treatments at the end of the experiment in 6 holes per treatment. Biosolid patches that remained in the planting holes and nearby soil were measured separately. Soil pH (0–30 cm depth) was determined from a 1:2.5 (w/v) solution of soil and deionized water, using the same soil samples.

2.4. *Seedling performance*

Survival and growth were recorded from February 2000 (at planting) to September 2004 on all plants. Root collar diameter and stem height were measured before and after summer during the first 2 years, and once in autumn afterwards.

Seedling water status and biomass accumulation were measured in July 2000, 5 months after planting, in 5 randomly selected plants per treatment. This evaluation was not performed in some treatments because of the initial low survival rates. However, we kept on measuring seedling performance in the H14 treatment with seedlings planted in the middle of the planting hole as a reference high mortality treatment.

We measured predawn water potential using a pressure bomb (Scholander et al., 1965) on 5 seedlings per treatment, and then we harvested the seedlings. Roots colonizing the soil were separated from those confined in the original root plug, and the entire root system was carefully washed. We dried all biomass fractions at 65 °C for 48 h and determined the dry weight. The aboveground fractions were digested in a heating block at 250 °C with a mixture of sulfuric acid and hydrogen peroxide (1:1, v/v) (Jones and Case, 1990). Total N was measured using the Kjeldahl method. Phosphorus, K, Cu, Ni and Zn were measured by ICP-OES (Perkin Elmer Optima 4300 Inductively Coupled Plasma–Optical Emission Spectrometry). In October 2000, 8 months after planting, we collected the needles and leaves from 5 randomly selected seedlings per treatment and analyzed foliar nutrient concentration as before.

During the destructive sampling, we measured the distance between the root plug and the biosolid patches that remained in the planting holes.

2.5. *Data analysis*

Survival curves were analyzed for each species using a Log Rank test (Pyke and Thompson, 1986). We first analyzed the survival response to the seedling location in the planting hole (center versus periphery), and with the location that released the highest survival rates, we then compared the treatment effect.

Soil properties, seedling growth, biomass accumulation and nutrition and water status were analyzed by using a one-way ANOVA for one fixed factor (treatment) with four levels. Differences between levels were compared by applying Tukey's *b* test at 0.05 significance level when ANOVA showed a significant treatment effect. Data were log-transformed when needed to ensure homoscedasticity. Root collar diameter in *P. halepensis* was analyzed by means of the Brown-Forsythe statistic as homoscedasticity could not be achieved. Due to the lack of sufficient alive individuals after the first summer in some treatments (mainly in seedling planted in the center of the hole), we discarded the seedling Location factor in the latter morphology data analysis, keeping only the peripheral-planted and control seedlings.

We used regression analysis to evaluate the degree of covariation between seedling performance and soil properties (EC and biosolid patches that remained in the planting holes). All statistical analyses were performed using the SPSS v.10.0 statistical package (SPSS Inc., Chicago, USA).

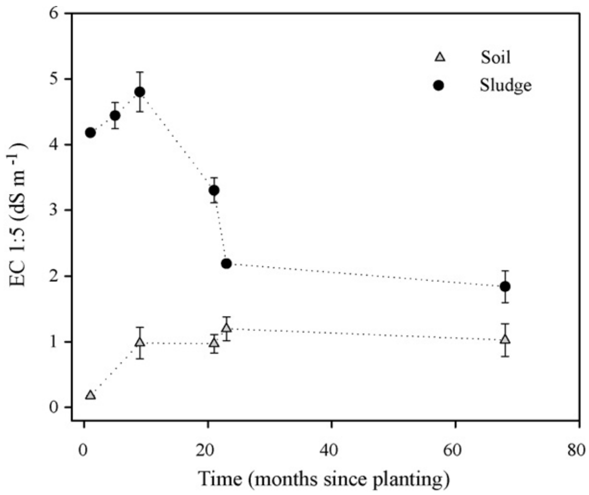

Fig. 1 – Electrical conductivity of the surface soil (0–30 cm depth) (triangles) and of the biosolid patches that remained in the planting hole (closed circles) in the H14 treatment for 5 years after planting. Data represent means and standard errors of $N = 10$.

3. Results

3.1. Soil properties

The soil surface area affected by biosolid application was higher in L14 than in H14 and H7, but maintained the same hole depth (35.5 ± 1.9, 36.3 ± 1.8 and 36.0 ± 2.5 cm, mean and standard error, respectively). The distribution of the biosolid in the planting hole ended up being quite heterogeneous and mainly concentrated in a 9–15 cm layer (Table 2).

During the first summer after plantation, 64, 47 and 40% of the holes in the L14, H14 and H7 treatments, respectively, showed cracks in the soil surface, caused by the drying and shrinking of sewage sludge within the planting holes. After the first summer, soil EC in treated soils ranged from 0.5 ± 0.1 dS m^{-1} in the upper soil layer to 4.6 ± 0.3 dSL m^{-1} in the 9–15 cm layer where most biosolids accumulated, with the deepest soil layers showing intermediate values (1.4 ± 0.1 dS m^{-1}). Electrical conductivity of biosolid aggregates increased during the first year reaching 4.8 ± 0.3 dS m^{-1}, and then decreased to 2.2 ± 0.1 dS m^{-1} after 20 months (Fig. 1), measured in the H14 treatment. Soil EC reached a maximum value of 1.2 ± 0.2 dS m^{-1} during the second year, and remained almost constant thereafter. Five years after biosolid application, soil in the H14 treatment showed higher EC (1.03 ± 0.25 dS m^{-1}; $F = 11.46$, $P < 0.001$) than soil in the control treatment (0.18 ± 0.01 dS m^{-1}), but was not significantly different from H7 (0.56 ± 0.15 dS m^{-1}) or L14 (0.69 ± 0.15 dS m^{-1}). The initial value of soil pH was 8.2 ± 0.1 and decreased to 7.8 ± 0.1 after the first summer, remaining constant thereafter (data not shown).

3.2. Seedling survival

P. halepensis and *Q. ilex* seedlings showed lower survival rates when planted in the center of the planting hole, as compared with the periphery location ($\chi^2 = 19.40$, d.f. = 1, $P < 0.001$ for *P. halepensis* and $\chi^2 = 30.01$, d.f. = 1, $P < 0.001$ for *Q. ilex*) (Fig. 2). When seedlings were peripherally planted, biosolids negatively affected the survival of *Q. ilex* seedlings as compared to control seedlings ($\chi^2 = 35.94$, d.f. = 3, $P < 0.001$). Results in *P. halepensis* showed a significant treatment effect ($\chi^2 = 13.41$, d.f. = 3, $P = 0.004$), and the pairwise comparison between curves showed that the application of 7.5 kg per hole (H7) did not affect the survival of *P. halepensis* as compared with control seedlings ($\chi^2 = 0.28$, $P = 0.59$). However, H7 enhanced seedling survival rate as compared with L14 and H14 ($P = 0.028$, $P = 0.078$, respectively). In both species, seedling mortality took place mainly during the first summer, with minor seedling death afterwards.

One year after planting, seedling survival was negatively related to soil EC in *P. halepensis*. Soil EC in the holes, where live seedlings were present, was 0.64 ± 0.21 dS m^{-1} in *P. halepensis* and 0.60 ± 0.16 dS m^{-1} in *Q. ilex*, whereas soil EC in the holes where seedlings were dead was 1.34 ± 0.17 dS m^{-1} and 1.45 ± 0.47 dS m^{-1} in *P. halepensis* ($P = 0.02$) and *Q. ilex* ($P = 0.29$), respectively. Five years after planting we found a negative relationship between soil EC and overall survival (including both seedling location, central and peripheral, in the planting hole) for both species (Fig. 3).

Crack-associated mortality increases ranged from 4 to 62% in *P. halepensis* and from 0 to 25% in *Q. ilex* (calculated as the difference in mortality between seedlings in planting holes presenting cracks, and in those that did not). Cracking mainly affected seedlings located in the middle of the planting hole and, especially, in point treatments (H).

3.3. Seedling growth

Stem height growth of *P. halepensis* seedlings receiving biosolids significantly increased as compared with control seedlings (Fig. 4). This difference vanished for L14 treatment after 5 years. Root collar diameter showed a similar pattern,

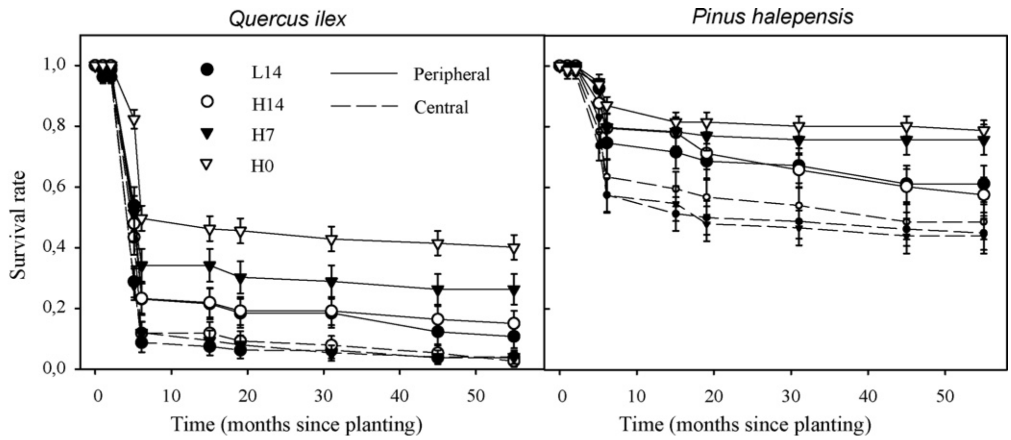

Fig. 2 – Survival rates of *Quercus ilex* (left) and *Pinus halepensis* (right) treated with different doses of biosolid and soil preparation and planted at two different locations, in the center and in the periphery of the planting hole. H0: control, H7: 7.5 kg d.w. planting hole^{-1}, H14: 14.5 kg d.w. planting hole^{-1}, L14: 14.5 kg d.w. planting hole^{-1} (enlarged holes). Data represent cumulative survival and standard error.

but only H14 seedlings were significantly bigger than control seedlings after 5 years. The H14 treatment showed higher relative height and diameter increment (22 and 23%, respectively) as compared with control seedlings. Aboveground growth of *Q. ilex* was very low, and was not affected by biosolid application.

During the first 5 months after planting, the biosolid application did not significantly affect shoot biomass accumulation in *Q. ilex* (Table 3). However, fine root biomass, length of roots colonizing the soil (fine roots) and rooting depth were significant and negatively affected by the highest dose, when seedlings were located in the center of the planting hole. We found a negative relationship between the root fraction and the distance of the root plug to the biosolid patches ($R^2 = 0.40$, $P = 0.01$, $N = 15$). As compared with control seedlings, *P. halepensis* seedlings increased aboveground biomass up to 80% with the higher dose when they were planted in the peripheral location. In this species, we found no effect of biosolid proximity on root biomass accumulation ($R^2 = 0.11$, $P = 0.17$, $N = 14$). Shoot:Root (S:R) index showed no significant effect of treatments, but S:R tended to increase in H7p and H14p treatments in both species.

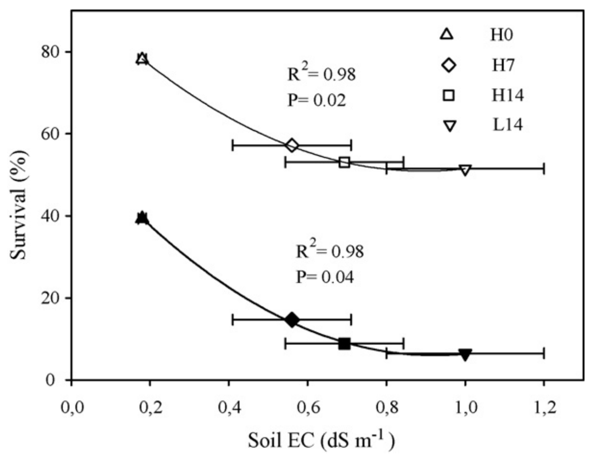

Fig. 3 – Relationship between soil electrical conductivity and seedling survival of *Pinus halepensis* (open symbols) and *Quercus ilex* (closed symbols) 5 years after planting. Survival percentage in each treatment includes central and peripheral location of seedlings. Soil EC was obtained from the complete homogenisation of the soil in the planting hole (0–30 cm depth). H0: control, H7: 7.5 kg d.w. planting hole^{-1}, H14: 14.5 kg d.w. planting^{-1}, L14: 14.5 kg d.w. planting hole^{-1} (enlarged holes). Data of soil EC represent means and standard errors of $N = 6$. Data were adjusted to quadratic equations.

3.4. *Water status and nutrient concentration*

During summer, the highest biosolid dose decreased the predawn water potential in *Q. ilex* seedlings planted in a central location, as compared with control plants (Table 4). We found a positive relationship between Ψ_w and the biomass of fine roots colonizing the soil in *Q. ilex*, but not in *P. halepensis* (Fig. 5). Water potential tended to increase in *P. halepensis* seedlings planted at the periphery of the planting hole as compared to control seedlings, but differences were not statistically significant. No differences in water potential were found in either of the two species after summer.

With biosolid application, *P. halepensis* needle N concentration increased in July when seedlings were planted in a peripheral location, as compared with control seedlings (Table 4). In contrast, P and K concentrations were significantly higher in control plants than in plants amended with biosolids; in the case of K, this is true only in plants amended with the highest dose and planted peripherally. Foliar N concentration in *Q. ilex* was significantly higher only in seedlings amended with the highest dose and placed in the center of the planting hole. Foliar K and P concentrations showed no significant treatment effect in this species. Only *P. halepensis* seedlings amended with the highest biosolid dose maintained

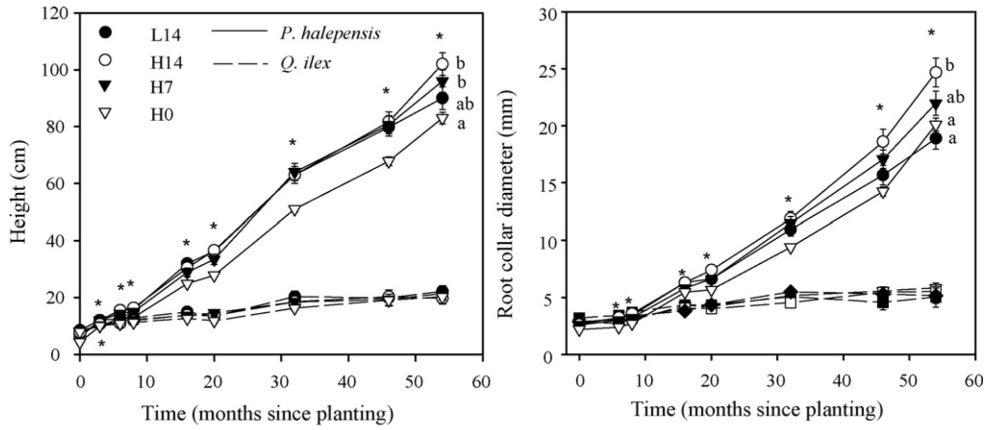

Fig. 4 – Stem height (left) and root collar diameter (right) in *Pinus halepensis* and *Quercus ilex* seedlings, as affected by different application rates of biosolid and soil preparation. Only seedlings planted in the periphery of the planting hole are shown. Symbols represent mean and standard errors of all alive individuals. See Fig. 2 for treatment explanation. Asterisks denote significant differences for a given sampling date ($P < 0.05$). Results of a Tukey's *b* test ($P < 0.05$) are indicated by letters in the last sampling date.

the differences in N concentration after summer. No differences in P and K concentrations were found for either of the two species after summer.

Foliar concentrations of Cu, Zn and Ni were low in both species at both sampling dates; in two cases they were below those of the control seedlings (Zn in July and Cu in October, in pine and oaks, respectively) (Table 4).

3.5. *Economic estimation*

It took an average of 40 s to dig each hole with the backhoe excavator. Taking into account biosolid loading and transport from the storage area (20–150 m from the planting holes), biosolid application, and mixing with the soil, the entire operation took on average 119–138 s hole^{-1}. Considering the cost of the machinery and personnel, biosolid application increased plantation costs by 77–89%, as compared with control plots.

4. Discussion

The application of anaerobically stabilized biosolids in the planting hole at the application rates used in this study had negative effects on seedling survival. These were associated with soil cracking and salinity increase during the first year while differences were maintained thereafter.

The viscous consistency of the biosolid resulted in a heterogeneous incorporation and a patchy distribution within the planting hole. At the beginning of the dry period, the rapid desiccation of these biosolid patches generates cavities within the planting hole, followed by surface cracks near the

Table 3 – Morphological traits of *Q. ilex* and *P. halepensis* seedlings measured during a destructive sampling in July 2000 (5 months after planting)

	Shoot (g)	Root (g)	Fine root (g)	Shoot:root	*D* (cm)[b]	*L* (cm)[b]
Q. ilex						
H0	2.0 ± 0.2	3.1 ± 0.4	0.16 ± 0.03a	0.66 ± 0.06	18 ± 4a	7 ± 1 ab
H7p	2.2 ± 0.4	3.1 ± 0.5	0.19 ± 0.07ab	0.73 ± 0.08	14 ± 3ab	12 ± 2 a
H14p[a]	2.0 ± 0.3	2.4 ± 0.2	0.12 ± 0.06ab	0.86 ± 0.12	9 ± 2ab	8 ± 1 ab
H14c[a]	1.7 ± 0.3	2.9 ± 0.5	0.03 ± 0.01b	0.63 ± 0.09	5 ± 3b	6 ± 1 b
F	0.30	0.63	3.38*	1.17	3.89**	4.34**
P. halepensis						
H0	1.5 ± 0.2a	1.2 ± 0.2	0.41 ± 0.08	1.53 ± 0.41	14 ± 4	25 ± 9
H7p	2.2 ± 0.1ab	1.2 ± 0.1	0.30 ± 0.07	1.80 ± 0.12	18 ± 5	26 ± 6
H14p	2.7 ± 0.5b	1.4 ± 0.2	0.39 ± 0.10	1.86 ± 0.26	18 ± 3	25 ± 8
H14c	2.0 ± 0.2ab	1.3 ± 0.1	0.29 ± 0.03	1.57 ± 0.16	15 ± 6	37 ± 6
F	2.65*	0.38	0.74	0.71	0.23	0.7

Mean and standard error of $N = 4$–5 seedlings, and results of one-way ANOVA are shown. Asterisks denote significant differences at the 0.05 (*) and 0.01 (**) significance levels. Results of a Tukey's *b* test ($P < 0.05$) are indicated by letters within the same column for each species.

[a] p and c indicate peripheral or central location of the seedlings in the planting hole.

[b] *D*, maximum rooting depth and *L*, maximum lateral root elongation.

Table 4 – Nutrient status and predawn water potential (ψ_w) of *Q. ilex* and *P. halepensis* seedlings measured during a destructive sampling in July 2000 (5 months after planting) and in October 2000 (8 months after planting)

	Q. ilex					*P. halepensis*				
	H0	H7p	H14p[a]	H14c[a]	F	H0	H7p	H14p[a]	H14c[a]	F
July										
N (%)	0.8 ± 0.1a	1.1 ± 0.2ab	1.2 ± 0.1ab	1.4 ± 0.2b	3.71*	1.0 ± 0.0a	1.6 ± 0.0b	1.4 ± 0.1b	1.3 ± 0.1ab	8.18**
P (%)	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.78	0.12 ± 0.01a	0.08 ± 0.00b	0.09 ± 0.01b	0.08 ± 0.00b	12.06**
K (%)	0.35 ± 0.01	0.35 ± 0.06	0.47 ± 0.04	0.52 ± 0.06	2.12	0.50 ± 0.03a	0.41 ± 0.04ab	0.38 ± 0.01b	0.42 ± 0.03ab	3.37*
Cu (mg kg^{-1})	1.8 ± 0.3	1.1 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	2.13	3.4 ± 0.3	3.2 ± 0.7	4.8 ± 1.2	3.0 ± 0.2	1.44
Ni (mg kg^{-1})	–	–	0.37 ± 0.32	–	–	–	–	0.38 ± 0.30	0.18 ± 0.13	–
Zn (mg kg^{-1})	9.9 ± 0.6	8.0 ± 1.2	10.3 ± 1.9	14.6 ± 4.6	1.06	28.5 ± 2.2a	20.3 ± 1.9b	21.0 ± 1.7b	18.4 ± 1.4b	6.07**
ψ_w(MPa)	−3.2 ± 0.6a	−3.8 ± 1.1ab	−5.5 ± 1.1ab	−6.8 ± 0.4b	3.44*	−2.9 ± 0.4	−2.1 ± 0.1	−2.2 ± 0.6	-3.1 ± 0.5	1.44
October										
N (%)	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	nd	2.45	1.0 ± 0.1a	1.2 ± 0.1ab	1.3 ± 0.1b	nd	3.8*
P (%)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	nd	0.03	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	nd	1.84
K (%)	0.30 ± 0.01	0.28 ± 0.03	0.26 ± 0.05	nd	0.41	0.32 ± 0.03	0.33 ± 0.03	0.35 ± 0.02	nd	0.42
Cu (mg kg^{-1})	2.5 ± 0.4a	2.1 ± 0.4ab	1.1 ± 0.1b	nd	3.92*	3.97 ± 0.2	5.7 ± 1.0	7.3 ± 1.5	nd	2.05
Ni (mg kg^{-1})	–	–	–	nd	–	0.7 ± 0.7	0.3 ± 0.1	–	nd	–
Zn (mg kg^{-1})	10.0 ± 0.6	12.3 ± 1.0	6.3 ± 1.0	nd	1.98	21.6 ± 4.1	16.8 ± 2.0	19.9 ± 2.6	nd	0.74
ψ_w(MPa)	−3.5 ± 0.3	−3.7 ± 0.9	−2.9 ± 0.8	nd	0.39	−1.4 ± 0.3	−1.4 ± 0.3	−0.9 ± 0.3	nd	0.74

Mean and standard error of $N = 4$–5 seedlings, and results of one-way ANOVA are shown. Asterisks denote significant differences at the 0.05 (*) and 0.01 (**) significance levels. Results of a Tukey's *b* test ($P < 0.05$) are indicated by letters within the same column for each species. nd, not determined; – below detection limit (<0.05).

[a] *p* and *c* indicate peripheral or central location of the seedlings in the planting hole.

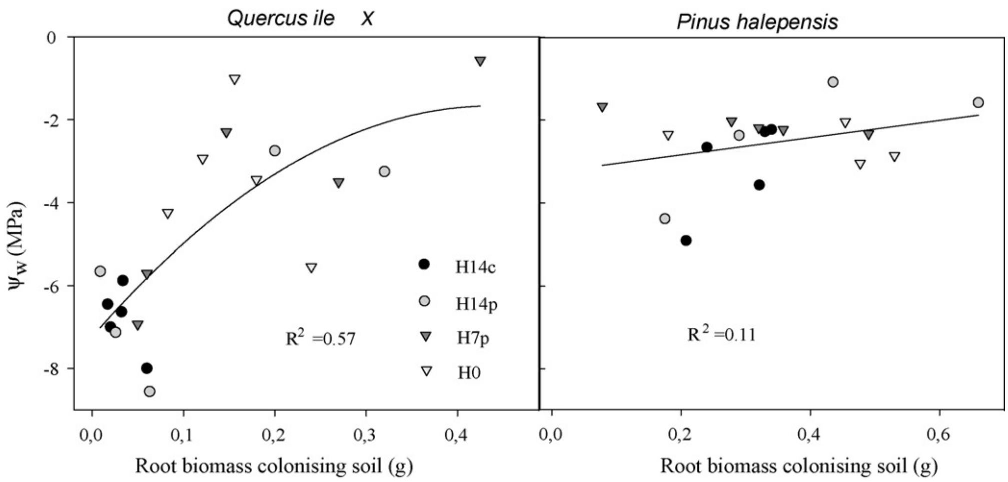

Fig. 5 – Relationship between the root biomass colonizing the planting hole and the predawn water potential of *Quercus ilex* (left) and *Pinus halepensis* (right) seedlings, unamended (H0) and treated with low (H7) and high (H14) doses of anaerobically stabilized sewage sludge applied at the planting hole. Seedlings were planted at two different locations, in the center (c) and in the periphery (p) of the planting hole. Data were adjusted to quadratic regression ($\Psi_w = -7.23 + 25.4x - 28.9x^2$; $F = 11.13$, $P < 0.000$) for *Q. ilex*, but were not significant for *P. halepensis* ($F = 1.8$, $P = 0.19$).

seedling root collar, that may negatively affect soil water storage (Bandyopadhyay et al., 2003), root contact with the soil, and thus seedling establishment.

The use of fresh biosolids as a soil amendment results in a high availability of soluble organic compounds with the potential to reactivate microbial activity and biochemical cycles in degraded soils faster than composted amendments (Pascual et al., 1999). But mineralization rates are usually higher in biosolids than in composted organic residues, mainly during the first year (Henry and Cole, 1997; Sort and Alcañiz, 1999; Sánchez-Monedero et al., 2004), and especially in sandy soils (Hernández et al., 2002). Low summer precipitation, 40% below the historical average (Ninyerola et al., 2005), may have favored salt concentration and intensified the negative effects of soil salinity. Seedling survival decreases related to increased salinity after biosolid application have been reported in other studies in dry Mediterranean areas (Ingelmo et al., 1998). Scarce information is currently available on the tolerance of *Q. ilex* and *P. halepensis* seedlings to soil salinity and its interaction with drought stress. *Quercus* species have been described as very sensitive to salinity (Thorton et al., 1988; Alaoui-Sossé et al., 1998), while conifers have been defined as sensitive (Moorhead and Ruter, 2002; Jacobs et al., 2004) or tolerant (Fostad and Pedersen, 2000) to soil salinity, depending on the size of the root stock and the methods used to determine tolerance (Jacobs and Timmer, 2005). During the first growing period, seedlings planted in the center of the planting hole showed symptoms of salt toxicity (desiccation of leaf margins and needle tips, reduced leaf size and early fall of nursery-grown leaves). These symptoms were more evident for *Q. ilex*. Moreover, the negative relationship between fine root accumulation in soil and the distance to biosolid patches found in *Q. ilex* seedlings suggests that root growth may have been negatively affected by soil salinity. Root growth inhibition is a common response to salinity, especially under dry conditions (Rodgers and Anderson, 1995; Jacobs et al., 2004), and it presents two different phases, one caused by the osmotic effect due to the salt accumulation outside the roots, and the other caused by internal injury when plants exceed the ability of the cells to compartmentalize salts in the vacuole (Munns, 2002). The decrease in water potential in *Q. ilex* seedlings, especially in those amended with the highest dose of biosolids and planted in the center of the planting hole, provides further support to the hypothesis that increased water stress resulting from higher osmotic potential may be the major driver for increased mortality in seedlings exposed to an increase of salinity levels in the rooting medium (Alonso et al., 2002; Jacobs et al., 2004; Óskarsson et al., 2006). It is worth noting that the water potential of the amended seedlings became similar to or lower than that of the unamended ones after the first September rains. The fast lateral root growth of *P. halepensis* seedlings shortly after planting may have alleviated the negative effect of increased salinity. Jacobs et al. (2003, 2004) observed injures in elongating root tips of *Pseudotsuga menziesii* in a highly fertilized soil layer, whereas roots proliferated in a more superficial layer that was less affected by salt accumulation. In our study, the negative effect of salinity was probably reduced by decreasing the biosolid dose and avoiding a direct early contact between the seedling and the biosolid (H7p treatment). Increased seedling mortality after biosolid application has frequently been attributed to higher competition with adventitious vegetation (Berry, 1977; Marx et al., 1995). In our study, the density of adventitious vegetation colonizing the planting hole was low during the first year, and mainly appeared during the second year (data not shown). By that time survival had stabilized, so competition from these plants was probably not a major driver of seedling mortality. We must take into account, however, that the pre-existing vegetation may have responded rapidly to biosolid application and soil disturbance by fostering root growth within the planting hole. Other studies have found significant belowground responses of Mediterranean vegetation to localized inorganic fertilizers (Valdecantos et al., 2006) and biosolid applications (Valdecantos, 2001).

A positive growth response of *P. halepensis* to organic amendments has been observed under Mediterranean climatic conditions (Roldan et al., 1996; Zagas et al., 2000; Valdecantos, 2001), and it has commonly been attributed to enhanced nutrient status. Biosolids rapidly increased the foliar N concentration of *P. halepensis* and *Q. ilex* by 43 and 54%, respectively. This probably favored the increase in aboveground biomass accumulation in amended *P. halepensis*, and the subsequent dilution of P and K. Koerselman and Meuleman (1996) determined that N:P ratios above 16 and below 14 may indicate P and N limitation, respectively. After the growing period, unamended plants of *P. halepensis* were strongly limited by N as the N:P ratio was 8.3 in front of the amended seedlings, that ranged between 15.5 (H14) and 19.8 (H7), and remained roughly constant after summer. This N limitation caused a lower growth rate in control seedlings, increasing the needle concentration of P (and K) as compared to reference values (Valdecantos et al., 2006). On the contrary, the increase in N concentration of amended seedlings of *Q. ilex* did not enhance plant growth since control plants showed relatively balanced N:P ratios (13.4 and 15.5 in July and October, respectively) in front of amended seedlings that increased up to 20. Usually, *Q. ilex* shows a low response to organic amendment applications (Alonso et al., 2002; Valdecantos, 2001; Larchevêque et al., 2006). Differences between *P. halepensis* and *Q. ilex* may be related to the contrasting strategies of both species. Pioneer species, such as *P. halepensis* in the study area, tend to keep foliar concentrations of the most limiting nutrient at relatively constant levels when its availability increases, using the extra nutrient inputs for increasing growth (Bazzaz, 1979). In contrast, late-successional species, like *Q. ilex* have a conservative resource-use strategy (Valladares et al., 2000), presenting low growth rates, and may allocate supplemental nutrient inputs to belowground storage organs (Rapp et al., 1999; Valdecantos, 2001). But in any case these adaptation strategies could be strongly dependent on environmental conditions (Broncano et al., 1998; Sardans and Peñuelas, 2004).

Nevertheless, the higher N uptake of amended plants could unbalance the N:P ratio, since we expected a higher response of foliar P concentration with the amendments (Díaz and Roldán, 2000; Sardans and Peñuelas, 2004; Sardans et al., 2006). Dry sites with highly carbonated soils could promote soil P immobilization (Smith, 1996; Tunesi et al., 1999; Rapp et al., 1999) and a subsequent decreased uptake by seedlings even after artificial P enrichment (Aerts and Chapin, 2000). Other factors could have immobilized a high amount of the total P added, such as the previous chemical treatment of the sewage sludge (Krogstad et al., 2005). The low heavy metal concentration found in aboveground tissues of treated seedlings (some of them lower than that found in control seedlings) confirmed the dilution effect caused by the increase in aboveground biomass, and probably the low seedling uptake and bioaccumulation of these elements in highly carbonated and alkaline soils on dry sites (Fuentes et al., 2007).

In our study, we purposely employed field machinery commonly used in forest practices so we could evaluate the logistic and economic aspects of biosolid application. The extra costs of applying biosolids were somewhat higher than those incurred for landfill disposal in the H7 treatment (+14%), but are lower than landfill disposal in the other treatments (−35%). We must take into account that the machinery used was not specifically designed to apply biosolids on rough terrain; thus, application costs could be substantially reduced by using self-loading spreaders. Thus, on-site logistic and economic factors do not seem to limit the use of this type of biosolids in degraded Mediterranean woodlands.

5. Conclusions

The use of high doses of anaerobically digested biosolids, calculated to combine economic and ecological feasibility, showed a negative effect on seedling survival, particularly at the highest doses. The higher mortality related to soil cracking may be avoided by affordable improvements in the application techniques that optimize the soil-biosolid mixing operation or by increasing biosolid quality (i.e., lower initial water content). Nevertheless, application rates should be decreased to avoid excessive salt accumulation near the seedling rhizosphere, which should not come into direct contact with the biosolid patches; this is especially true in the case of *Q. ilex* whose rooting strategy (depth vs. lateral growth) may increase its limitations for colonizing non-amended soil. On the other hand, using application doses below the ones we assessed may improve seedling survival but increase operational costs compromising the global viability.

Our findings of improved nutrient status and growth in *P. halepensis* seedlings, together with balanced economic costs, suggest that biosolids can be used to improve the establishment of this species in degraded shrublands. In contrast, *Q. ilex* showed a negative response to biosolid application, so the use of this technique for the establishment of *Q. ilex* cannot be recommended under the conditions of the present study. Further studies must be carried out to establish optimal application rates and distances so that seedlings do not sustain either phytotoxic effects or additional drought stress from the decreased water availability produced by salt concentration in the seedling rhizosphere.

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