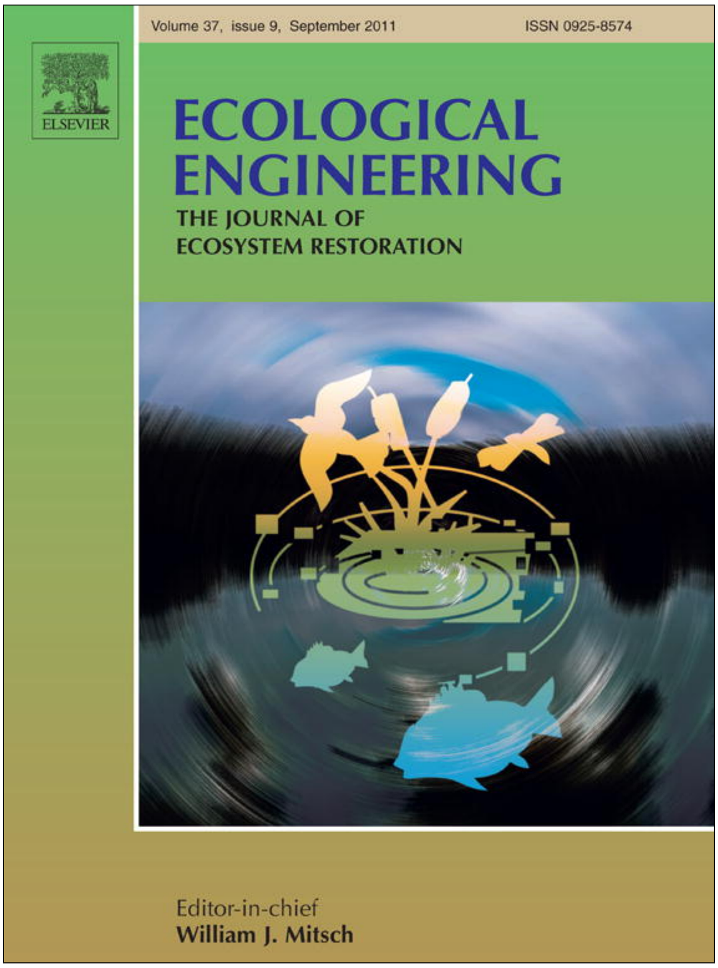
Volume 37, issue 9, September 2011
ISSN 0925-8574
ELSEVIER
ECOLOGICAL ENGINEERING
THE JOURNAL OF ECOSYSTEM RESTORATION
Editor-in-chief
William J. Mitsch

Ecological Engineering 37 (2011) 1350–1359

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Differential field response of two Mediterranean tree species to inputs of sewage sludge at the seedling stage

Alejandro Valdecantos [a,*], Jordi Cortina [b], V. Ramón Vallejo [a]

[a] Centro de Estudios Ambientales del Mediterráneo (CEAM), C/Charles R. Darwin 14, Parque Tecnológico, 46980 Paterna, Spain
[b] Departamento de Ecología, Universidad de Alicante, Ap. 99, 03080 Alicante, Spain

ARTICLE INFO

Article history:
Received 5 November 2010
Received in revised form 8 March 2011
Accepted 27 March 2011
Available online 22 April 2011

Keywords:
Biomass allocation pattern
Biosolids
Foliar isotopic composition
Pinus halepensis
Quercus ilex
Restoration
Vector diagnose

ABSTRACT

Land degradation and desertification is a common feature in Mediterranean landscapes due to extensive and intensive land use and natural or man induced disturbances. The ecosystem may need external inputs to recover its composition and function as soils are often impoverished and vegetal key stone species lost. We evaluated the effects of the application of fresh and air-dried biosolids in the establishment and morphological and physiological performance of seedlings of *Pinus halepensis* and *Quercus ilex* under dry Mediterranean field conditions. Seedling survival was not affected by biosolid treatments in any of the studied species both two and ten years after planting. During the first two years, growth was enhanced by the two biosolid treatments in relation to control, although the change in the biomass allocation pattern differed between species. Rooting depth was significantly enhanced by liquid biosolid in *Q. ilex* and marginally reduced in *P. halepensis* as well as the exploration of soil. As a consequence, root-to-shoot ratio reduced significantly with dry and liquid sludge due to promoted aboveground growth while maintaining and even reducing belowground fractions. An improvement of the nutritional status, of fertilized seedlings especially of phosphorus, is the explanation for the better field performance. Vector analysis revealed an important phosphorus limitation for both species that was overcome with the application of liquid (both species) and air-dried biosolid (pine). The higher growth of pine seedlings attained in the liquid biosolid treatment was coupled with a significant decrease in foliar $\delta^{13}C$, suggesting lower water use efficiency. The significant increase in foliar $\delta^{15}N$ in the biosolid treatments in both species suggested that a large proportion of the total nitrogen uptake came from the applied biosolids. Instead, with regard to the low biosolid application rate used in the study, treatments had an overall positive effect as a restoration tool by improving nutritional status and promoting growth of planted seedlings.

1. Introduction

Mediterranean landscapes have been extensively modified by land use (Grove and Rackham, 2001). Over the last decades, forest plantations and spontaneous colonization of marginal agricultural land may have favored the increase in forest cover (either woodland or shrubland) and fire occurrence (Romero-Calcerrada and Perry, 2004; Vega-García and Chuvieco, 2006). When a disturbance such as a fire event takes place, the recovery of pre-disturbance ecosystem integrity may be too slow for society's needs, and favor further negative landscape processes such as erosion (Pausas et al., 2008). Under these circumstances, restoration actions have been recommended to foster the establishment of keystone woody species, reassemble forest communities and improve ecosystem functioning (Cortina et al., 2006; Vallejo et al., 2006).

Degraded Mediterranean soils are often impoverished in organic matter and nutrients (Martínez-Mena et al., 2002; Alguacil et al., 2009) and the establishment of tree seedlings can be hampered by low availability of soil resources (Grogan et al., 2003; Valdecantos et al., 2006). The application of organic amendments may improve soil fertility and water availability, promote biological activity and facilitate seedling establishment (Henry et al., 1994; García et al., 2000; Querejeta et al., 2001; Larcheveque et al., 2006a). Sewage sludge has been widely used in agriculture and commercial forestry, but its use for the restoration of degraded Mediterranean areas is scarce and mostly restricted to the forestation of abandoned agricultural fields and severely damaged areas such as minesites (Navas et al., 1999; Brofas et al., 2000; Jorba and Andrés, 2000; Ojeda et al., 2003). A few experimental applications of sewage sludge in burned areas suggest that they can be used to foster the establishment of target species (Larcheveque et al.,

* Corresponding author. Tel.: +34 96 590 9731, fax: +34 96 590 9825.
E-mail addresses: a.valdecantos@ua.es (A. Valdecantos), jordi@ua.es (J. Cortina), ramonv@ceam.es (V.R. Vallejo).

0925-8574/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ecoleng.2011.03.017

2006a, 2010; Fuentes et al., 2010). However, degraded rangelands differ from crops and old-fields because accessibility is commonly lower, topography steeper and uneven, and soils are shallower and stony. In addition, vegetation cover and the level of ecosystem functioning are higher in degraded rangelands, and it may be valuable to preserve and use them to improve restoration success (Gomez-Aparicio et al., 2005; Cortina et al., 2006). Differences in land use and degree of ecosystem damage constrain management options for sewage sludge application and affect their costs (Fuentes et al., 2007a).

In areas subjected to seasonal drought, seedling response to sewage sludge may be further affected by its interaction with water availability and the negative consequences related to drought overcome the positive ones derived from organic amendments (Larcheveque et al., 2010). Sewage sludge may contain water and help seedlings to cope with transplant shock and reach deep soil horizons before the onset of summer drought (O'Dell and Claasen, 2006; O'Dell et al., 2007). Organic amendments may also increase water storage capacity and water availability by modifying soil structure and infiltration rate (Querejeta et al., 2000). Conversely, sewage sludge may increase soluble salt content and promote physiological drought (Fuentes et al., 2007b). Finally, increased nutrient availability may induce changes in seedling morpho-physiological traits that may either favor (Trubat et al., 2006) or compromise (Graciano et al., 2006) seedling ability to withstand drought. The isotopic composition of plant tissues is an interesting tool in order to indirectly assess plant functioning in an integrate way. Foliar $\delta^{13}C$ has been proposed as an analogue variable to integrated water use efficiency (WUE) (Farquhar et al., 1989) as it integrates photosynthesis rates since leaf outbreak. On the other hand, the depuration process of wastewaters produces enriched ^{15}N biosolids as by-product (Wang et al., 2004). The assessment of foliar $\delta^{15}N$ represents an approach to determine the amount of absorbed N coming from the organic amendments (Querejeta et al., 2008).

Seedlings of tree species respond to changes in resource availability in contrasting ways, depending on life traits and resource demands. For instance, Canham et al. (1996) found that at different light levels, *Pinus strobus* seedlings responded positively to increased amounts of water and nutrients, whereas in the same experiment, increased nitrogen supply did not promote growth in *Quercus rubra* seedlings. Species also differ in their ability to allocate biomass belowground in response to changes in soil resource availability and the magnitude of change in allocation pattern is related to the degree of tolerance to other aboveground disturbances and resources (Portsmuth and Niinemets, 2007). Similarly, species of fertile sites reduce the root:shoot ratio with nutrient enrichment more intensely than species typical of low fertility sites (Lusk et al., 1997). Shallow-rooting Mediterranean species are more sensitive to increased soil fertility than deep-rooting species (Larcheveque et al., 2010).

Pinus halepensis and *Quercus ilex* are among the most frequently used tree species for the restoration of degraded areas in the Western Mediterranean basin (Pausas et al., 2004). Planting success is highly heterogeneous for both species, depending on site quality, interspecific interactions, climatic conditions and seedling quality (Castro et al., 2004; Del Campo et al., 2007; Valdecantos et al., 2009). Survival of *P. halepensis* seedlings is commonly higher than that of *Q. ilex*, and may be substantially improved by soil preparation techniques aimed at increasing soil fertility and water availability (Querejeta et al., 2001; Navarro Cerrillo et al., 2005; Del Campo et al., 2006). *Q. ilex* is a relatively drought-tolerant species as it keeps stomata open under mild drought (Damesin et al., 1998). Comparatively, *P. halepensis* behaves as a drought-avoiding species, showing early stomatal closure and residual transpiration (Levitt, 1980; Borghetti et al., 1998; Villar-Salvador et al., 2005).

Table 1
Physical features and soil properties of the three planting sites.

	Planting site		
	Bolinches	Cabello	Gachas
Latitude	39°00′N	38°59′N	39°01′N
Longitude	0°54′W	0°57′W	0°55′W
Elevation (m a.s.l.)	950	1000	950
Total plant cover (%)	66	71	61
pH (H_2O)	8.4	8.0	8.3
Organic matter (%)	1.6	4.3	3.7
Total N (%)	0.10	0.21	0.16
Total CO_3 (%)	37.4	10.8	29.9
P available (ppm)	4.3	8.1	6.3

Both *P. halepensis* and *Q. ilex* are sensitive to nutrient inputs (Sardans et al., 2006), but their response to the application of organic amendments at planting depends on amendment type and dose, and soil and climatic conditions. So, *P. halepensis* responds rapidly to organic amendments (Valdecantos et al., 1996; Barberá et al., 2005; Fuentes et al., 2010) and inorganic fertilization (Sardans et al., 2005). In contrast, *Q. ilex* response to fertilization is highly heterogeneous, ranging from reductions to increases in growth rate (Seva et al., 1996; Pardos et al., 2005; Sardans et al., 2006). Commonly, the effect of enhanced nutrient supply on *Q. ilex* is lower than the effect on *P. halepensis* (Broncano et al., 1998; Larcheveque et al., 2006b)

Recommendations on the use of sewage sludge in the restoration of degraded Mediterranean rangelands are thus hampered by the interactions between sludge, soil and plants. More information is needed to understand the effects of sewage sludge on the establishment of tree species before they can be efficiently used at a management level. With this aim, we carried out a field experiment to evaluate the response of seedlings of *P. halepensis* and *Q. ilex* to sewage sludge application, and explore the drivers of such response. Our hypotheses are that (1) sewage sludge application improves seedling survival and growth, modifying biomass allocation patterns, and (2) the magnitude of these responses is higher in *P. halepensis* than in *Q. ilex*.

2. Materials and methods

2.1. Study area

Three experimental plots were located in an inland area in Ayora (Valencia, Eastern Spain) at an altitude of 950–1000 m a.s.l. (Table 1). The area has dry meso-Mediterranean climate (357 mm and 15.1 °C total annual precipitation and mean monthly temperature, respectively; 1990–2000 Ayora weather station, Confederación Hidrográfica del Júcar). Plot size is 0.3 ha, and plots are more than 2 km away from each other. Plots were located in a flat area (5–16% slope), on shallow, alkaline soils, developed from marl. Soil organic matter contents and available P are low, especially in Bolinches site (Table 1), but however they fall within the lowest part of the range found in the bibliography (Lucas-Borja et al., 2010). Since 1979, the area was affected by several wildfires, the last one occurring before the onset of the study in 1991. A low and sparse shrubland (less than 45 cm height and 61–71% plant cover) dominated by obligate seeder species such as *Rosmarinus officinalis* L., *Ulex parviflorus* (Pourr.), and *Cistus* spp. cover the area. Spontaneous recruitment of *P. halepensis* and *Q. ilex* is scarce.

Table 2
Main physico-chemical properties of the sewage sludge used in the experiment and limit values for concentrations of heavy metals in sludge for use on land established by the EU Directive 86/278/EEC.

	Sewage sludge applied	Directive 86/278/EEC
Ashes (%)	71	
Organic matter (%)	29	
Total N (%)	5.6	
Total P (%)	2.0	
Fe (ppm)	3938	
Cu (ppm)	220	1000–1750
Zn (ppm)	803	2500–4000
Ni (ppm)	16	300–400
Cd (ppm)	0.3	20–40
Cr (ppm)	27	–
Pb (ppm)	68	750–1200
Hg (ppm)	<0.05	16–25

2.2. Experimental design and seedling performance

A 20 cm diameter by 40 cm depth auger was used to dig planting holes in February 1997. The experimental treatments included the application liquid sewage sludge (LS), air-dried sewage sludge (DS), and no amendment (Control; CO). Sludge was produced in a nearby domestic waste water treatment plant and showed low contents of heavy metals (Table 2). Dry biosolids were obtained by air drying sewage water on sand beds until moisture content was ca. 15%. Liquid biosolid (1.4% dry matter) was applied at the bottom of the planting holes before planting, whereas air-dried biosolid was deposited on the topsoil surrounding the planting holes after they were filled. Application rate was equivalent to 10 Mg dry weight ha^{-1} for both amendments. In spring 1997, 50 (control and liquid biosolid) or 25 (dry biosolid) one-year old seedlings of *P. halepensis* Mill. and *Q. ilex* L. were planted on separate holes in each experimental plot (i.e., 125 seedlings per species and plot).

Seedling stem height and root collar diameter were measured in all surviving individuals every two months from March 1997 to October 1998 (except in November 1997). A final survivorship analysis was carried out in December 2006. In October 1998, we conducted a destructive sampling of five seedlings per species and treatment (total $n = 30$) in one of the sites (Gachas). We carefully excavated the root systems and measured maximum rooting depth and lateral expansion, to infer the soil volume explored by roots. Seedlings were taken to the lab, separated into above and belowground fractions, washed with distilled water and oven-dried at 60 °C for 48 h. Subsamples of current-year leaves and needles were counted and weighed to calculate mean leaf and needle weight, and analyzed for nutrient content and concentration. Ground samples were digested with concentrated H_2SO_4 and H_2O_2 (30%, v/v). We determined N concentration by using semi-micro-Kjeldahl distillation (Tecator Kjeltec Auto 1030 Analyzer, Hogana, Sweden), and P and K concentration by ICP spectrometry (Perkin Elmer Optima 3000, Perkin Elmer Corp., Norwalk, CT, USA). Leaf and needle nutrient content was estimated as the product of foliar dry weight by nutrient concentration. We applied vector analysis (Weetman, 1989) to visualize the effect of biosolid application on nutritional status.

Ground foliar tissues from five seedlings of each species X treatment combination were analyzed for ^{15}N and ^{13}C by continuous-flow direct combustion and mass spectrometry using a Europa Scientific SL-2020 system (Stable Isotope Lab, Utah State University, Logan, UT, USA). Results are expressed as $\delta^{15}N$ and $\delta^{13}C$ relative to the atmospheric N_2 and Pee Dee Belemnite (PDB). These isotopic analyses were used to evaluate integrated water use efficiency (^{13}C) and the use of N derived from the biosolid (^{15}N; Wang et al., 2004; Querejeta et al., 2008). We estimated the proportion of foliar N supplied by the biosolid by using the equation by Powlson and Barraclough (1993):

$$F = \frac{T(A_S - A_B)}{A_F}$$

where F is the weight of foliar N derived from the biosolid, T is the foliar N content, and A is the atom percentage excess of the foliar sample of amended seedlings ($_S$), control seedlings ($_B$) and the biosolid ($_F$). As A_F we took a value of +15 (Heaton, 1986).

Table 3
Survival (%) of *Pinus halepensis* and *Quercus ilex* seedlings 20 months and ca. ten years after planting in a degraded Mediterranean area as affected by biosolid application (mean and standard error of $n = 3$ planting sites).

Treatment	*Pinus halepensis*		*Quercus ilex*	
	October 1998	December 2006	October 1998	December 2006
Control	97 (2)	74 (14)	88 (6)	43 (16)
Air-dried biosolid	99 (2)	70 (13)	96 (2)	51 (12)
Liquid biosolid	93 (7)	64 (13)	91 (7)	48 (7)

2.3. Data analysis

The effect of biosolid application and planting site on survival rates was evaluated using log-linear models (χ^2). Changes in stem height and root collar diameter of the surviving individuals at the end of the experiment were log-transformed to homogenize variances and analyzed by repeated measures ANOVA with one within subjects factor (time), and two between subjects factors (biosolid application and planting site) at three levels each. Because the sphericity assumption was not met, the Greenhouse–Geisser correction was used to adjust the degrees of freedom (Ott, 1988). Biomass accumulation and allocation patterns, maximum rooting depth and extension, and nutrient concentration and contents and foliar carbon and nitrogen isotopic composition were analyzed by one-way ANOVA (with biosolid application as a fixed factor at three levels). Nutrient concentrations were log-transformed when needed to avoid heteroscedasticity. Pairwise comparisons were performed by using Tukey's HSD test when ANOVA results showed significant factor effects. All analyses were carried out by using SPSS v.15.0 statistical package (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Seedling performance

Seedling survival after the second summer in the field was higher than 88% (Table 3) and was not affected by biosolid application ($\chi^2 = 4.21$, $p = 0.239$ and $\chi^2 = 3.65$, $p = 0.301$ for *Q. ilex* and *P. halepensis*, respectively). Planting site affected the establishment of both species ($\chi^2 = 24.14$, $p < 0.0001$, and $\chi^2 = 36.13$, $p < 0.0001$ for *Q. ilex* and *P. halepensis*, respectively). No site x treatment interaction was observed in any of the two species. Ten years after planting, survivorship in *P. halepensis* ranged between 64 and 74% while in *Q. ilex* was between 43 and 51%. We did not observe effects of the biosolid treatment in any of the two species ($\chi^2 = 4.55$, $p = 0.103$ and $\chi^2 = 0.91$, $p = 0.635$ for *P. halepensis* and *Q. ilex*, respectively). The effect of the planting site was still significant in the survival rates of both species ($\chi^2 = 53.03$, $p < 0.0001$, and $\chi^2 = 39.96$, $p < 0.0001$ for *P. halepensis* and *Q. ilex*, respectively). A significant planting site x treatment interaction was recorded in holm oak survival ($\chi^2 = 10.43$, $p = 0.034$) as biosolid application significantly improved the survival rates in Cabello. This is the site that showed the lowest

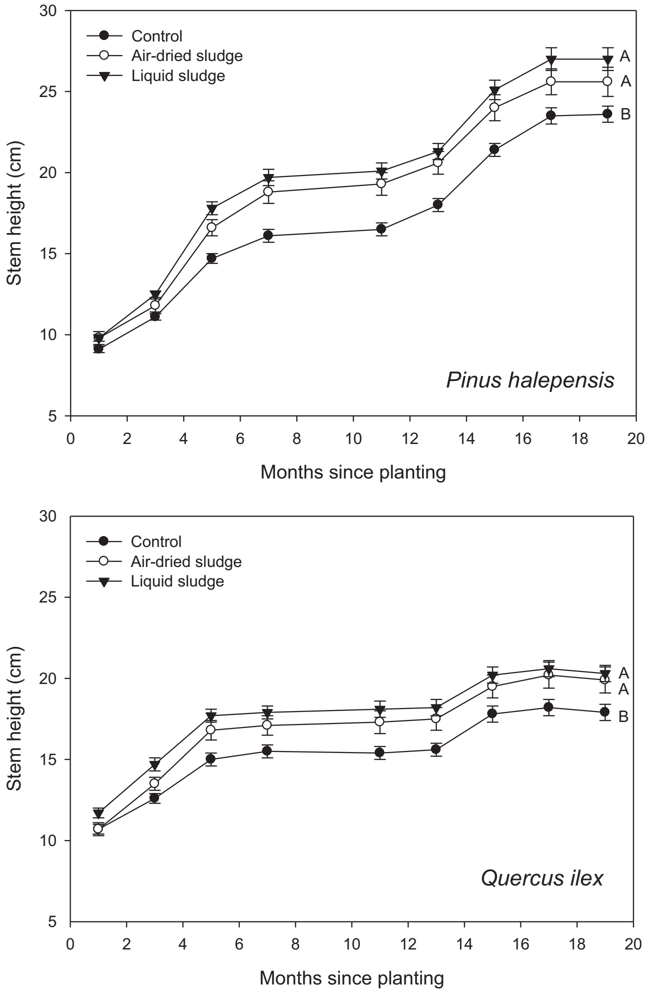

Fig. 1. Changes in stem height of *Pinus halepensis* (left) and *Quercus ilex* (right) seedlings as affected by liquid and air-dried biosolid (means and standard errors are shown). Seedlings were planted in February 1997. Different letters indicate significant differences in repeated-measures ANOVA ($p < 0.05$).

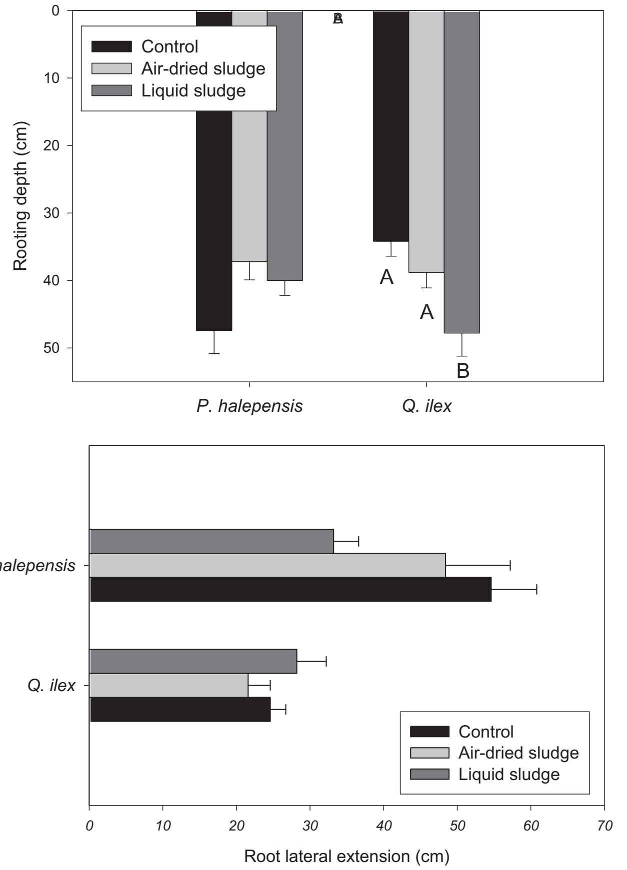

Fig. 2. Maximum rooting depth (left) and maximum root lateral extension (right) of *Pinus halepensis* and *Quercus ilex* seedlings 20 months after planting as affected by biosolid application (mean and standard error of $n = 5$ seedlings). Different letters for a given species correspond to significant differences between treatments (Tukey HSD, $p < 0.05$).

survival percentages (11.4, 28.0 and 34.0% in control, air-dried and liquid sludge, respectively, in December 2006).

Stem height of *P. halepensis* and *Q. ilex* was higher in seedlings receiving biosolids than in unamended seedlings (Fig. 1, Table 4). Results for basal diameter were similar (data not shown). For example, pines amended with liquid biosolid showed 6 and 11% increases in stem height and root collar diameter, respectively, as compared to unamended seedlings. The planting site showed a significant effect on seedling performance but species differed in their response. The site showing highest growth rates for *P. halepensis* was Gachas, and the one showing the lowest was Cabello. In contrast, *Q. ilex* showed higher basal diameter in Cabello than in Gachas and Bolinches. The effect of biosolid application on seedling growth changed through time, as seedling size was promoted during the first growing season due to treatments and no further increase was recorded thereafter.

3.2. Root growth and biomass partitioning

Two summers after planting, maximum rooting depth was almost 40% higher in *Q. ilex* seedlings receiving liquid biosolid at the bottom of the planting hole than in unamended seedlings (Fig. 2). Root lateral extension was slightly higher in seedlings receiving liquid biosolid and lower in those receiving air-dried biosolid (27.8 *vs* 22.4 cm, respectively, $F = 1.09$, $p = 0.354$). Maximum rooting depth of *P. halepensis* seedlings showed no significant response to biosolid application, but amended seedlings showed a marginally significant trend towards shallower roots that remained closer to the root plug (33.2 *vs* 54.6 cm of lateral extension in liquid biosolid and control seedlings, respectively, $F = 2.84$, $p = 0.098$).

Aboveground biomass of pines amended with liquid sludge was significantly higher than in control seedlings (Table 5). The same trend was observed in oaks, but differences were not statistically significant. We found no effect of biosolid application on belowground biomass accumulation. As a result, the root-to-shoot ratio showed a sharp reduction with the application of organic amendments in *P. halepensis*, but not in *Q. ilex*.

3.3. Nutritional status

Foliar nitrogen concentration was always below 10 mg g^{-1} and 7 mg g^{-1} in *P. halepensis* and *Q. ilex*, respectively. Liquid biosolid increased P concentration in *P. halepensis* seedlings by more than 2-fold, whereas in *Q. ilex* seedlings increased from 0.58 to 0.78 mg g^{-1} (Table 6). Mean leaf weight in *Q. ilex* seedlings amended with dry and liquid sludge was 66 and 90% higher, respectively, than in control seedlings. Foliar P content was also higher in amended seedlings than in control seedlings. Other nutrients showed the same trend but differences were not statistically significant. Vector analysis showed that unamended *P. halepensis* seedlings were limited by phosphorus, but nitrogen and potassium were sufficient,

Table 4
Results of the repeated measures ANOVA to evaluate the effect of biosolid application and planting site on stem height of *Pinus halepensis* and *Quercus ilex* seedlings.

Between subjects	*Pinus halepensis*			*Quercus ilex*		
	df	*F*	*p*	df	*F*	*p*
Biosolid (B)	2	15.29	<0.001	2	8.52	<0.001
Site (S)	2	43.91	<0.001	2	2.33	0.099
B × S	4	0.65	0.628	4	0.79	0.534
Error	311			313		

Within subjects	*Pinus halepensis*				*Quercus ilex*			
	df	*F*	*p*	G-Gp	df	*F*	*p*	G-Gp
Time	8	2051.7	<0.001	<0.001	8	661.9	<0.001	<0.001
Time × B	16	4.2	<0.001	0.003	16	2.6	0.001	0.033
Time × S	16	9.2	<0.001	<0.001	16	4.8	<0.001	<0.001
Time × B × S	32	1.5	0.032	0.151	32	1.6	0.023	0.124
Error	2488				2504			

G-Gp: *p* value after applying the Greenhouse–Geisser estimation by adjusting the degrees of freedom.

Table 5
Biomass partitioning of *Pinus halepensis* and *Quercus ilex* seedlings 20 months after planting as affected by biosolid application (mean and standard error of $n = 5$ seedlings, and results of the one-way ANOVA). Different letters for a given species correspond to significant differences between treatments (Tukey HSD, $p < 0.05$). R:S = root-to-shoot ratio.

		Aboveground biomass (g)	Belowground biomass (g)	Total biomass (g)	R:S
Pinus halepensis	Control	15.2 (0.5)a	9.2 (0.6)	24.4 (0.5)	0.61 (0.05)a
	Air-dried sludge	17.2 (2.1)ab	7.3 (0.9)	24.5 (3.0)	0.43 (0.02)b
	Liquid sludge	22.2 (2.2)b	9.0 (0.7)	31.2 (2.5)	0.42 (0.05)b
		$F = 4.047$	$F = 1.939$	$F = 2.993$	$F = 6.311$
		$p = 0.045$	$p = 0.186$	$p = 0.088$	$p = 0.013$
Quercus ilex	Control	5.6 (0.2)	9.3 (0.9)	14.9 (0.9)	1.66 (0.19)
	Air-dried sludge	8.2 (1.8)	11.2 (2.5)	19.5 (4.1)	1.41 (0.22)
	Liquid sludge	10.6 (1.9)	13.3 (1.1)	23.9 (2.9)	1.37 (0.19)
		$F = 2.571$	$F = 1.433$	$F = 1.501$	$F = 0.600$
		$p = 0.118$	$p = 0.277$	$p = 0.262$	$p = 0.565$

and did not limit seedling growth (Fig. 3). Phosphorus was also limiting for *Q. ilex* seedlings. The decrease in foliar K concentration with an increase in foliar K content and mean leaf weight suggests a dilution of this nutrient in *Q. ilex* seedlings after liquid biosolid application.

3.4. Isotopic composition of leaves and needles

Values of $\delta^{13}C$ varied within a narrow range (<1‰) in each species. Despite of this, differences between the two types of biosolid application were significant in *P. halepensis* ($F = 4.08$, $p = 0.045$); seedlings amended with air-dried biosolid showed higher water use efficiency than those receiving liquid biosolid (Table 7). Amended *Q. ilex* seedlings showed a trend towards higher water use efficiency than unamended ones, but it was not statistically significant.

Biosolid application, especially when applied in liquid form, significantly increased $\delta^{15}N$ in both species ($F = 69.02$, $p < 0.0001$ and $F = 22.10$, $p < 0.0001$ in *P. halepensis* and *Q. ilex*, respectively; Table 7). We estimated that 54–68% of foliar N of *Q. ilex* seedlings was supplied by the biosolid (air-dried and liquid biosolid, respectively) whereas the proportion of the foliar N derived from biosolids was 46 and 44% in *P. halepensis* receiving air-dried and liquid biosolid, respectively.

4. Discussion

4.1. Seedling survival

Seedling establishment was very successful in the first two years, especially for *Q. ilex*. Early seedling mortality in this species

Table 6
Leaf and needle nutrient concentration and weight of *Pinus halepensis* and *Quercus ilex* seedlings 20 months after planting as affected by biosolid application (mean and standard error of $n = 5$ seedlings, and results of the one-way ANOVA). Different letters for a given species correspond to significant differences between treatments (Tukey HSD, $p < 0.05$).

		N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Mean leaf weight (mg)
Pinus halepensis	Control	9.8 (0.8)	1.1 (0.1)a	2.4 (0.2)	3.73 (0.61)
	Air-dried sludge	9.2 (0.8)	1.9 (0.2)b	2.7 (0.3)	4.47 (0.65)
	Liquid sludge	9.5 (0.4)	2.5 (0.2)c	2.4 (0.3)	4.36 (0.72)
		$F = 0.228$	$F = 16.896$	$F = 0.282$	$F = 0.364$
		$p = 0.800$	$p = 0.000$	$p = 0.759$	$p = 0.702$
Quercus ilex	Control	6.5 (0.4)	0.6 (0.1)	4.7 (1.6)	31.7 (6.4)
	Air-dried sludge	6.8 (0.5)	0.6 (0.0)	5.1 (0.7)	52.7 (9.7)
	Liquid sludge	6.6 (0.2)	0.8 (0.1)	2.8 (0.4)	60.4 (16.5)
		$F = 0.123$	$F = 1.859$	$F = 1.407$	$F = 1.637$
		$p = 0.886$	$p = 0.198$	$p = 0.283$	$p = 0.235$

Table 7
Carbon and nitrogen isotopic composition of needles of *Pinus halepensis* and leaves of *Quercus ilex* seedlings as affected by biosolid application. Means and standard error of $n = 5$ seedlings are shown. Different letters for a given species correspond to significant differences between treatments (Tukey HSD, $p < 0.05$).

		$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Pinus halepensis	Control	−25.7 (0.2)ab	−5.4 (0.4)a
	Air-dried sludge	−25.5 (0.3)b	0.7 (0.5)b
	Liquid sludge	−26.4 (0.2)a	2.2 (0.6)b
Quercus ilex	Control	−28.5 (0.6)	−6.7 (0.9)a
	Air-dried sludge	−27. 8 (0.4)	−1.7 (0.7)b
	Liquid sludge	−27.7 (0.7)	2.0 (1.2)c

can be very high, even in set-aside agricultural areas with deep soils (Villar-Salvador et al., 2004; Rey Benayas et al., 2005). Mortality of *P. halepensis* seedlings can also be high, although it is usually lower than for *Q. ilex* (Valdecantos et al., 2006; Del Campo et al., 2007). However, survival in the long term fell to around 70 and 50% in Aleppo pine and holm oak, respectively, but these figures are often found in the literature (Fuentes et al., 2007a). For instance, Rey Benayas (1998) recorded survival rates around 50% three years after planting *Q. ilex* seedlings in a set-aside agricultural field, and Pausas et al. (2004) reported survival rates 7.5 years after planting between 40 and 60% for both Aleppo pine and holm oak seedlings planted in soils developed form marls as in our study. The three selected planting sites share many features, like fire and land use history, geomorphology, and vegetation composition and cover. But despite their apparent uniformity, seedling survival and growth differed significantly among the three planting sites. None of the site properties measured could explain these differences, which may result from subtle differences in climate and/or soil properties as observed in other studies (Maestre et al., 2003). Despite the significance of this factor, we found no interaction between site and sewage sludge application for seedling survival and growth, indicating that the effect of sewage sludge did not depend on site conditions within the range of variability of the present study.

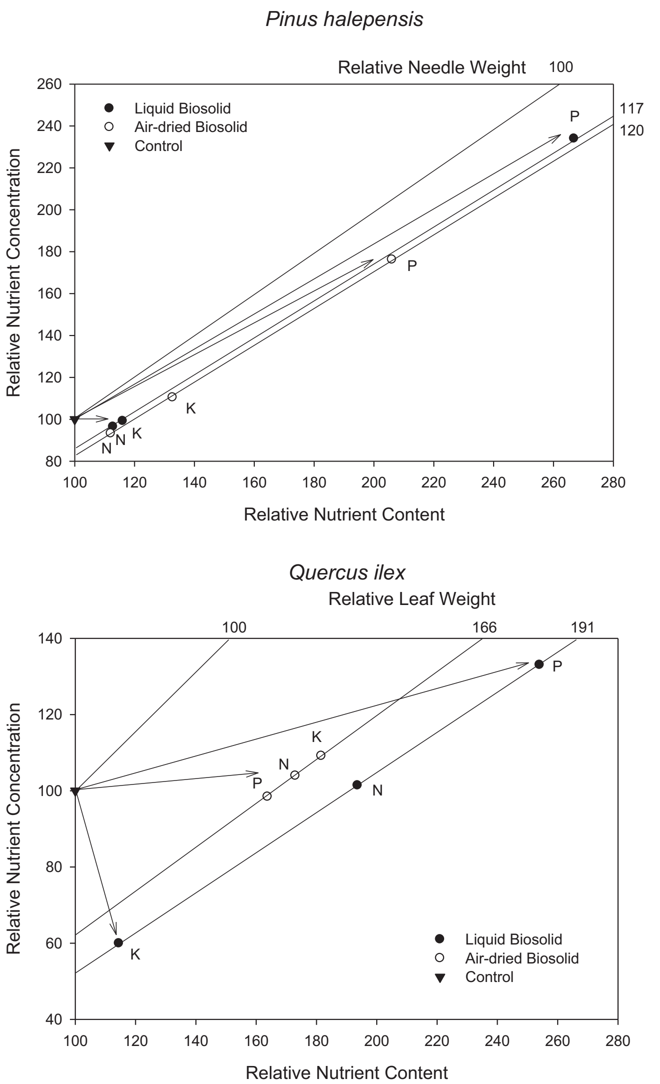

Fig. 3. Graphical vector analysis describing the response of *Pinus halepensis* and *Quercus ilex* seedlings to biosolid application. Data from control seedlings (black triangles) were used as reference (value of 100 for average leaf or needle weight, nutrient content and concentration). They correspond to 9.8 mg N g^{-1}, 1.1 mg P g^{-1}, 2.4 mg K g^{-1}, 3.7 mg of needle weight for *P. halepensis*, and 6.5 mg N g^{-1}, 0.6 mg P g^{-1}, 4.7 mg K g^{-1}, 31.7 mg of leaf weight for *Q. ilex*. Diagonal isolines correspond to foliar dry weight.

Sewage sludge application has shown positive, negative or no effect on seedling survival (Sheedy, 1997; Larcheveque et al., 2006b; Fuentes et al., 2007a,b, 2010). Contrasting results may arise from differences in application rate, species sensitivity to nutrient availability and soil salinity, and soil and climate conditions. As mentioned before, survival rates were high in unamended seedlings, suggesting that growing conditions during the two years after planting were relatively mild. Thus, they probably promoted positive effects of sewage sludge application and reduced negative ones, such as those derived from increased soil salinity (Fuentes et al., 2010). In addition, application rates in the present study were low as compared to other studies (Román et al., 2003; Larcheveque et al., 2006a, 2010; Fuentes et al., 2007a, 2010), probably reducing the risk of increased soluble salt content in the soil.

4.2. Seedling growth, biomass allocation and soil colonization

The effects of organic amendments on plant growth may last, at least, as much as those obtained by commercial inorganic fertilizers (Zasoski et al., 1983; Miller, 1990; Hasselgren, 1998). Studies on the application of sewage sludge at planting in Mediterranean areas are scarce (Valdecantos et al., 2004). As for survival, there are evidences of increased and decreased seedling growth after sewage sludge application (Larcheveque et al., 2006b; Fuentes et al., 2007a). Negative effects of sewage sludge on seedling growth are commonly associated to high application rates. As a general rule, rates above 40 Mg ha^{-1} are not recommended in this type of application (Fuentes et al., 2010), a rate that is well above the one used in the present study. Other organic amendments, such as urban solid wastes, commonly promote seedling growth, an effect that has been attributed to improved soil water holding capacity and soil fertility (García et al., 2000; Querejeta et al., 2001; Caravaca et al., 2002, 2003; Barberá et al., 2005).

The increase in the amount of biomass allocated belowground is a common response to low nutrient availability (Poot and Lambers, 2003). In contrast, changes in water availability commonly result in weak alterations of biomass allocation patterns (Poot and Lambers, 2003; Cortina et al., 2008). Our results suggest that the application of sewage sludge increased soil resource availability, to the point of reducing biomass allocation belowground in *P. halepensis*. The trend was similar in *Q. ilex*, but it was not statistically significant. Results of the foliar nutrient analysis and $\delta^{13}C$ data support this observation, and suggest that P deficiency, which is common in highly carbonated soils (Carreira et al., 1997; Valdecantos et al., 2006), was the main nutrient deficiency alleviated by sewage sludge.

The increase in rooting depth found in sludge-amended seedlings of *Q. ilex* did not affect survival rates in the field. However, it may affect future ability to withstand drought, as rooting depth is strongly related to seedling survival under Mediterranean conditions (Lloret et al., 1999; Padilla and Pugnaire, 2007). *Q. ilex* develops a taproot with a marked orthogeotropic development (Pemán et al., 2006), even after nursery pruning (J. Monerris, Univ. of Alicante, unpubl. data). Biosolids, when applied at the bottom of the planting hole, promoted this growing pattern by increasing nutrient availability or reducing soil resistance to penetration.

The soil volume explored by roots as defined by lateral extension and rooting depth was more than 3 fold higher in unamended *P. halepensis* seedlings than in those amended with liquid sewage sludge. Seedling root density was probably higher when seedlings received liquid sewage sludge, as total belowground biomass was similar in all cases. Increases in root density after the application of organic amendments have been described elsewhere (Mou et al., 1997).

4.3. Nutrient uptake and status

Sewage sludge application had a strong effect on P nutrition of both species studied. In contrast, its effect on nitrogen status was never significant. This does not mean that N was not supplied by sewage sludge or used by growing seedlings, as shown by isotopic measurements. Organic refuses usually contain high ^{15}N content, and $\delta^{15}N$ enrichment of leaves and needles can be used as a tracer of N added with the biosolids (Wang et al., 2004). In our study, foliar N isotopic composition was sensitive to the application of sewage sludge, as $\delta^{15}N$ substantially increased in amended seedlings as compared to unamended ones. Similar results have been observed after the application of liquid biosolids in *Pinus radiata* plantations in New Zealand (Wang et al., 2005) and composted urban refuse in *P. halepensis* plantations in SE Spain (Querejeta et al., 2008), suggesting that a substantial amount of plant N uptake originates from organic amendments. We estimated that up to 2/3 of the foliar N of *P. halepensis* and *Q. ilex* was sourced from biosolids. Spontaneous vegetation probably benefited from nutrient inputs in the sewage sludge (Wang et al., 2005), although the localized application may have reduced this impact as compared to broadcast applications.

Fertilization frequently results in a decrease in foliar concentration of those nutrients that are added at a lower rate, and may lead to nutrient imbalances (George and Seith, 1998; Fuentes et al., 2007a). The observed reduction in K concentration of *Q. ilex* leaves after liquid biosolid application was probably the result of dilution, as leaf growth was promoted while little extra K was supplied because the depuration process removes large quantities of K from sewage sludge (Mengel and Kirkby, 1987). This effect has been observed after inorganic P fertilization of adult *Q. ilex* (Sabaté and Gracia, 1994).

4.4. Water use efficiency

The $\delta^{13}C$ values of *P. halepensis* needles were lower than those reported in the literature for this species (Peñuelas et al., 1999; Klein et al., 2005; Querejeta et al., 2008) but similar to those observed under irrigation (Klein et al., 2005). Needle carbon isotopic composition of this species is sensitive to increases in soil water availability, with decreases of up to 4‰ of $\delta^{13}C$ when supplemented with water (Klein et al., 2005). This is above the change observed in the present study (i.e., <1‰). But despite the small change in ^{13}C induced by sewage sludge application, differences between seedlings receiving liquid and air-dried sewage sludge were significant, suggesting that liquid sewage sludge decreased water use efficiency (low stomata control) and/or a higher water stress in air-dried sludge amended seedlings (stomata closure). This may be a direct result of irrigation at planting, or an indirect effect of changes in biomass allocation and nutritional status. It is interesting to note that *P. halepensis* seedlings receiving liquid and air-dried sewage sludge did not differ in biomass accumulation, biomass allocation or rooting depth, but only in needle P concentration. As a general trend, increased nutrient availability (Fernández et al., 2006; Brueck et al., 2010) and leaf P concentration (Querejeta et al., 2003) has been related to higher water use efficiency, a trend that was not supported by our data.

Damesin et al. (1998) observed that a decrease in minimum predawn water potential of −4 MPa in *Q. ilex* and *Q. pubescens* would induce an increase of 0.7‰ in $\delta^{13}C$. It has been proposed that shallow-rooting species develop higher water use efficiency during drought periods than deep-rooting species (Valentini et al., 1992). But our results do not support a clear relationship between rooting depth and water use efficiency. We observed that less negative $\delta^{13}C$ values were recorded in those treatments that reached deeper soil horizons, i.e. control for *P. halepensis* and liquid sewage sludge for *Q. ilex*. But treatment effects were not statistically significant for rooting depth in *P. halepensis* and isotopic composition in *Q. ilex*. Differences in rooting depth between treatments (≈10 cm) may not be sufficient to affect water use efficiency at this stage.

4.5. Interspecific comparison

Both species studied were sensitive to sewage sludge application and showed similar responses to the experimental treatments by increasing seedling growth and phosphorus uptake. The sensitivity of these species to increased phosphorus availability has been reported in previous works (Sardans et al., 2004, 2006). Larcheveque et al. (2006b) related the increase in stem height of *P. halepensis* and *Q. ilex* seedlings after the application of composted sewage sludge to improved nutritional status. In general, *P. halepensis* is more plastic than *Q. ilex* as it shows larger changes in growth, physiological traits and nitrogen uptake and retranslocation in response to biotic and abiotic stresses (Cuesta et al., 2010) and prioritizes C allocation to aboveground growth (Sanz-Perez et al., 2009). However, although *P. halepensis* was more sensitive to treatments than *Q. ilex*, we observed a high degree of plasticity in both species: promoted seedling growth, differential belowground development with biosolids, change in the biomass allocation pattern, and improved P nutrition. It is interesting to note the contrasting effect of biosolid application on the rooting pattern of both species. Holm oak seedlings used the extra resources provided by biosolids (especially in liquid form) to build up deeper rooting systems, whereas in pines root biomass accumulation and rooting depth was not affected by the amendment. This contrasting behavior may reflect differences in ecological strategies and life traits between *P. halepensis* and *Q. ilex*, which are located in opposite sides of the competition *vs* stress tolerance gradient (sensu Grime, 1977).

4.6. Management implications

Sewage sludge application had a positive effect on seedling nutrient status and growth, and no effect on seedling survival, resulting in a weak increase in basal area. In addition, deleterious effects of sewage sludge application on the environment and nearby communities were minimized by using small application rates and confining the amendment to planting holes. The cost of sewage sludge application in management operations is highly dependent on the type of sewage sludge, availability of suitable equipment, site conditions and transportation (Tarrason et al., 2007). Commonly, it represents a 2-fold increase in the cost of planting which may only be compensated when other costs, such as landfill dumping, are taken into account (Fuentes et al., 2007a). Thus, the use of sewage sludge may only be justified as an alternative to other uses, provided that the return of organic matter and nutrients to degraded ecosystems is of interest.

5. Conclusions

We have shown that the application of sewage sludge at planting improves seedling growth by alleviating P deficiency in Mediterranean carbonated soils and, in the case of liquid sludge, by improving water status. These results support our first hypothesis. In contrast, *P. halepensis* and *Q. ilex* showed contrasting changes in morpho-functional traits after sewage sludge application, but results did not support our second hypothesis of a stronger response of the coniferous species. The benefits of sewage sludge at this application rate on seedling establishment were modest but alleviated nutrient limitation and promoted seedling growth.

Acknowledgements

We appreciate the contribution of three anonymous referees that improved the quality of the original manuscript. This research was conducted in the framework of the projects RECUVES (077/RN08/04.1) and BIOMET (608/2006/1-4.4) funded by the Spanish Ministry of Environment, and GRACCIE (CSD2007-00067, CONSOLIDER-INGENIO 2010 Program). Fundación CEAM is supported by Generalitat Valenciana and Fundación Bancaja.

References

Alguacil, M.M., Diaz-Pereira, E., Caravaca, F., Fernandez, D.A., Roldan, A., 2009. Application of organic amendments to a semiarid degraded soil increases AM fungi diversity in a long-term field experiment. Appl. Environ. Microbiol. 75, 4254–4263.

Barberá, G.G., Martínez-Fernández, F., Álvarez-Rogel, J., Albaladejo, J., Castillo, V., 2005. Short- and intermediate-term effects of site and plant preparation techniques on reforestation of a Mediterranean semiarid ecosystem with *Pinus halepensis* Mill. New Forests 29, 177–198.

Borghetti, M., Cinnirella, S., Magnani, F., Saracino, A., 1998. Impact of long-term drought on xylem embolism and growth in *Pinus halepensis* Mill. Trees 12, 187–195.

Brofas, G., Michopoulos, P., Alifragis, D., 2000. Sewage sludge as an amendment for calcareous bauxite mine spoils reclamation. J. Environ. Qual. 29, 811–816.

Broncano, M.J., Riba, M., Retana, J., 1998. Seed germination and seedling performance of two Mediterranean tree species, holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Mill.): a multifactor experimental approach. Plant Ecol. 138, 17–26.

Brueck, H., Erdle, K., Gao, Y., Giese, M., Zhao, Y., Peth, S., Lin, S., 2010. Effects of N and water supply on water use-efficiency of a semiarid grassland in Inner Mongolia. Plant Soil 328, 495–505.

Canham, C.D., Berkowitz, A.R., Kelly, V.R., Lovett, G.M., Ollinger, S.V., Schnurr, J., 1996. Biomass allocation and multiple resource limitation in tree seedlings. Can. J. Forest Res. 26, 1521–1530.

Caravaca, F., Barea, J.M., Roldán, A., 2002. Synergistic influence of an arbuscular mycorrhizal fungus and organic amendment on *Pistacia lentiscus* L. seedlings afforested in a degraded semiarid soil. Soil Biol. Biochem. 34, 1139–1145.

Caravaca, F., Figueroa, D., Azcón-Aguilar, C., Barea, J.M., Roldán, A., 2003. Medium-term effects of mycorrhizal inoculation and composted municipal waste addition on the establishment of two Mediterranean shrub species under semiarid field conditions. Agr. Ecosyst. Environ. 97, 95–105.

Carreira, J.A., Lajtha, K., Niell, F.X., 1997. Phosphorus transformations along a soil/vegetation series of fire-prone, dolomitic, semi-arid shrublands of southern Spain. Biogeochemistry 39, 87–120.

Castro, J., Zamora, R., Hodar, J.A., Gomez, J.M., Gomez-Aparicio, L., 2004. Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4-year study. Restor. Ecol. 12, 352–358.

Cortina, J., Maestre, F.T., Vallejo, R., Baeza, M.J., Valdecantos, A., Perez-Devesa, M., 2006. Ecosystem structure, function, and restoration success: are they related? J. Nat. Conserv. 14, 152–160.

Cortina, J., Green, J.J., Baddeley, J.A., Watson, C.A., 2008. Root morphology and water transport of *Pistacia lentiscus* seedlings under contrasting water supply: a test of the pipe stem theory. Environ. Exp. Bot. 62, 343–350.

Cuesta, B., Villar-Salvador, P., Puértolas, J., Jacobs, D.F., Rey Benayas, J.M., 2010. Why do large, nitrogen rich seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting Mediterranean forest species. Forest Ecol. Manag. 260, 71–78.

Damesin, C., Rambal, S., Joffre, R., 1998. Seasonal and annual changes in leaf $\delta^{13}C$ in two co-occurring Mediterranean oaks: relations to leaf growth and drought progression. Funct. Ecol. 12, 778–785.

Del Campo, A., Navarro, R.M., Aguilella, A., Gonzalez, E., 2006. Effect of tree shelter design on water condensation and run-off and its potential benefit for reforestation establishment in semiarid climates. Forest Ecol. Manag. 235, 107–115.

Del Campo, A.D., Navarro Cerrillo, R.M., Hermoso, J., Ibáñez, A.J., 2007. Relationships between site and stock quality in *Pinus halepensis* Mill. reforestation on semiarid landscapes in eastern Spain. Ann. Forest Sci. 64, 719–731.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Phys. 40, 503–537.

Fernández, M., Novillo, C., Pardos, J.A., 2006. Effects of water and nutrient availability in *Pinus pinaster* Ait. Open pollinated families at an early age: growth, gas exchange and water relations. New Forests 31, 321–342.

Fuentes, D., Disante, K.B., Valdecantos, A., Cortina, J., Vallejo, V.R., 2007a. Response of *Pinus halepensis* Mill. seedlings to biosolids enriched with Cu, Ni and Zn in three Mediterranean forest soils. Environ. Pollut. 145, 316–323.

Fuentes, D., Valdecantos, A., Cortina, J., Vallejo, V.R., 2007b. Seedling performance in sewage sludge-amended degraded Mediterranean woodlands. Ecol. Eng. 31, 281–291.

Fuentes, D., Valdecantos, A., Llovet, J., Cortina, J., Vallejo, V.R., 2010. Fine-tuning of sewage sludge application to promote the establishment of *Pinus halepensis* seedlings. Ecol. Eng. 36, 1221.

García, C., Hernández, T., Roldán, A., Albaladejo, J., Castillo, V., 2000. Organic amendment and mycorrhizal inoculation as a practice in afforestation of soils with *Pinus halepensis* Miller: effect on their microbial activity. Soil Biol. Biochem. 32, 1173–1181.

George, E., Seith, B., 1998. Long-term effects of a high nitrogen supply to soil on the growth and nutritional status of young Norway spruce trees. Environ. Pollut. 102, 301–306.

Gomez-Aparicio, L., Zamora, R., Gomez, J.M., 2005. The regeneration status of the endangered *Acer opalus* subsp. *granatense* throughout its geographical distribution in the Iberian Peninsula. Biol. Conserv. 121, 195–206.

Graciano, C., Guiamet, J.J., Goya, J.F., 2006. Fertilization and water stress interactions in young *Eucalyptus grandis* plants. Can. J. Forest Res. 36, 1028–1034.

Grime, J.P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111, 1169–1194.

Grogan, J., Ashton, M.S., Galvao, J., 2003. Big-leaf mahogany (*Swietenia macrophylla*) seedling survival and growth across a topographic gradient in southeast Pará, Brazil. Forest Ecol. Manag. 186, 311–326.

Grove, A.T., Rackham, O., 2001. The Nature of Mediterranean Europe. An Ecological History. Yale University Press, London.

Hasselgren, K., 1998. Use of municipal waste products in energy forestry: highlights from 15 years of experience. Biomass Bioenerg. 15, 71–74.

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem. Geol. 59, 87–102.

Henry, C.L., Cole, D.W., Harrison, R.B., 1994. Use of municipal sludge to restore and improve site productivity in forestry: the Pack Forest sludge research program. Forest Ecol. Manag. 66, 137–149.

Jorba, M., Andrés, P., 2000. Effects of sewage sludge on the establishment of the herbaceous ground cover after soil restoration. J. Soil Water Conserv. 55, 322–327.

Klein, T., Hemming, D., Lin, T., Grünzweig, J.M., Maseyk, K., Rotenberg, E., Yakir, D., 2005. Association between tree-ring and needle d13C and leaf gas exchange in *Pinus halepensis* under semi-arid conditions. Oecologia 144, 45–54.

Larcheveque, M., Baldy, V., Montès, N., Fernandez, C., Bonin, G., Ballini, C., 2006a. Short-term effects of sewage-sludge compost on a degraded Mediterranean soil. Soil Sci. Soc. Am. J. 70, 1178–1188.

Larcheveque, M., Ballini, C., Korboulewsky, N., Montes, N., 2006b. The use of compost in afforestation of Mediterranean areas: effects on soil properties and young tree seedlings. Sci. Total Environ. 369, 220–230.

Larcheveque, M., Ballini, C., Baldy, V., Korboulewsky, N., Ormeño, E., Montes, N., 2010. Restoration of a Mediterranean postfire shrubland: plant functional responses to organic soil amendment. Restor. Ecol. 18, 729–741.

Levitt, J., 1980. Responses of Plants to Environmental Stresses. Academic Press, New York.

Lloret, F., Canovas, C., Penuelas, J., 1999. Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio, seed size and water and nitrogen use. Funct. Ecol. 13, 210–216.

Lucas-Borja, M.E., Bastida, F., Nicolás, C., Moreno, J.L., del Cerro, A., Andrés, M., 2010. Influence of forest cover and herbaceous vegetation on the microbiological and biochemical properties of soil under Mediterranean humid climate. Eur. J. Soil Biol. 46, 273–279.

Lusk, C.H., Contreras, O., Figueroa, J., 1997. Growth, biomass allocation and plant nitrogen concentration in Chilean temperate rainforest tree seedlings: effects of nutrient availability. Oecologia 109, 49–58.

Maestre, F.T., Cortina, J., Bautista, S., Bellot, J., Vallejo, R., 2003. Small-scale environmental heterogeneity and spatiotemporal dynamics of seedling establishment in a semiarid degraded ecosystem. Ecosystems 6, 630–643.

Martínez-Mena, M., Alvarez Rogel, J., Castillo, V., Albaladejo, J., 2002. Organic carbon and nitrogen losses influenced by vegetation removal in a semiarid Mediterranean soil. Biogeochemistry 61, 309–321.

Mengel, K., Kirkby, E.A., 1987. Principles of Plant Nutrition. International Potash Institute (Publ.), Bern, Switzerland.

Miller, H.G., 1990. Management of water and nutrient relations in European forests. Forest Ecol. Manag. 30, 425–436.

Mou, P., Mitchell, R.J., Jones, R.H., 1997. Root distribution of two tree species under a heterogeneous nutrient environment. J. Appl. Ecol. 34, 645–656.

Navarro Cerrillo, R.M., Fragueiro, B., Ceaceros, C., del Campo, A., de Prado, R., 2005. Establishment of *Quercus ilex* L. subsp. *ballota* [Desf.] Samp. using different weed control strategies in southern Spain. Ecol. Eng. 25, 332–342.

Navas, A., Machín, J., Navas, B., 1999. Use of biosolids to restore the natural vegetation cover on degraded soil in the badlands of Zaragoza (NE Spain). Bioresour. Technol. 69, 199–205.

O'Dell, R.E., Claasen, V.P., 2006. Vertical distribution of organic amendment influences the rooting depth of revegetation species on barren, subgrade serpentine substrate. Plant Soil 285, 19–29.

O'Dell, R., Silk, W., Green, P., Claassen, V., 2007. Compost amendment of Cu–Zn minespoil reduces toxic bioavailable heavy metal concentrations and promotes establishment and biomass production of *Bromus carinatus* (Hook and Arn.). Environ. Pollut. 148, 115–124.

Ojeda, G., Alcañiz, J.M., Ortiz, O., 2003. Runoff and losses by erosion in soils amended with sewage sludge. Land Degrad. Dev. 14, 563–573.

Ott, L., 1988. An Introduction to Statistical Methods and Data Analysis, 3rd edition. PWS-Kent Publishing Company, Boston, USA.

Padilla, F.M., Pugnaire, F.I., 2007. Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Funct. Ecol. 21, 489–495.

Pardos, M., Royo, A., Pardos, J.A., 2005. Growth, nutrient, water relations, and gas exchange in a holm oak plantation in response to irrigation and fertilization. New Forests 30, 75–94.

Pausas, J.G., Bladé, C., Valdecantos, A., Seva, J.P., Fuentes, D., Alloza, J.A., Vilagrosa, A., Bautista, S., Cortina, J., Vallejo, R., 2004. Pines and oaks in the restoration of Mediterranean landscapes of Spain: new perspectives for an old practice – a review. Plant Ecol. 171, 209–220.

Pausas, J.G., Llovet, J., Rodrigo, A., Vallejo, R., 2008. Are wildfires a disaster in the Mediterranean basin? – a review. Int. J. Wildland Fire 17, 713–723.

Pemán, J., Voltas, J., Gil-Pelegrín, E., 2006. Morphological and functional variability in the root system of *Quercus ilex* L. subject to confinement: consequences for afforestation. Ann. For. Sci. 63, 425–430.

Peñuelas, J., Filella, I., Terradas, J., 1999. Variability of plant nitrogen and water use in a 100-m transect of a subdesertic depression of the Ebro valley (Spain) characterized by leaf $\delta^{13}C$ and $\delta^{15}N$. Acta Oecol. 20, 119–123.

Poot, P., Lambers, H., 2003. Are trade-offs in allocation pattern and root morphology related to species abundance? A congeneric comparison between rare and common species in the south-western Australia flora. J. Ecol. 91, 58–67.

Portsmuth, A., Niinemets, U., 2007. Structural and physiological plasticity in response to light and nutrients in five temperate deciduous woody species of contrasting shade tolerance. Funct. Ecol. 21, 61–77.

Powlson, D.S., Barraclough, D., 1993. Mineralization and assimilation in soil-plant systems. In: Knowles, R., Blackburn, T.H. (Eds.), Nitrogen Isotope Techniques. Academic Press Inc, San Diego, pp. 209–242.

Querejeta, J.I., Roldán, A., Albaladejo, J., Castillo, V., 2000. Soil physical properties and moisture content affected by site preparation in the afforestation of a semiarid rangeland. Soil Sci. Soc. Am. J. 64, 2087–2096.

Querejeta, J.I., Roldán, A., Albaladejo, J., Castillo, V., 2001. Soil water availability improved by site preparation in a *Pinus halepensis* afforestation under semiarid climate. Forest Ecol. Manag. 149, 115–128.

Querejeta, J.I., Barea, J.M., Allen, M.F., Caravaca, F., Roldán, A., 2003. Differential response of d13C and water use efficiency to arbuscular mycorrhizal infection in two aridland woody plant species. Oecologia 135, 510–515.

Querejeta, J.I., Barbera, G.G., Granados, A., Castillo, V.M., 2008. Afforestation method affects the isotopic composition of planted *Pinus halepensis* in a semiarid region of Spain. Forest Ecol. Manag. 254, 56–64.

Rey Benayas, J.M., 1998. Growth and survival in *Quercus ilex* L. seedlings after irrigation and artificial shading on Mediterranean set-aside agricultural land. Ann. Sci. Forest 55, 801–807.

Rey Benayas, J.M., Navarro, J., Espigares, T., Nicolau, J.M., Zavala, M.A., 2005. Effects of artificial shading and weed mowing in reforestation of Mediterranean abandoned cropland with contrasting *Quercus* species. Forest Ecol. Manag. 212, 302–314.

Román, R., Fortún, C., García López de Sá, M.E., Almendros, G., 2003. Successful soil remediation and reforestation of a calcic regosol amended with composted urban waste. Arid Land Res. Manag. 17, 297–311.

Romero-Calcerrada, R., Perry, G.L.W., 2004. The role of land abandonment in landscape dynamics in the SPA 'Encinares del río Alberche y Cofio, Central Spain, 1984–1999. Landscape Urban Plan. 66, 217–232.

Sabaté, S., Gracia, C.A., 1994. Canopy nutrient content of a *Quercus ilex* L. forest: fertilization and irrigation effects. Forest Ecol. Manag. 68, 31–37.

Sanz-Perez, V., Castro-Diez, P., Joffre, R., 2009. Seasonal carbon storage and growth in Mediterranean tree seedlings under different water conditions. Tree Physiol. 29, 1105–1116.

Sardans, J., Penuelas, J., Rodà, F., 2005. Changes in nutrient use efficiency, status and retranslocation in young post-fire regeneration *Pinus halepensis* in response to sudden N and P input, irrigation and removal of competing vegetation. Trees 19, 233–250.

Sardans, J., Rodà, F., Peñuelas, J., 2006. Effects of a nutrient pulse supply on nutrient status of the Mediterranean trees *Quercus ilex* subsp. *ballota* and *Pinus halepensis* on different soils and under different competitive pressure. Trees 20, 619–632.

Sardans, J., Rodà, F., Peñuelas, J., 2004. Phosphorus limitation and competitive capacities of *Pinus halepensis* and *Quercus ilex* subsp. *rotundifolia* on different soils. Plant Ecol. 174, 307–319.

Seva, J.P., Vilagrosa, A., Valdecantos, A., Cortina, J., Vallejo, V.R., Bellot, J., 1996. Mycorhhization et application de compost urbain pour l'amélioration de la survie et de la croissance des semis de *Quercus ilex* ssp. *ballota* en milieu sec. Options Mediterraneennes 20, 105–121.

Sheedy, G., 1997. Quelques résultats préliminaires de croissance d'une plantation d'épinette blanche traitée avec des boues de station d'épuration. Note Recherche Forestière 78, 1–11.

Tarrason, D., Ortiz, O., Alcaniz, J.M., 2007. A multi-criteria evaluation of organic amendments used to transform an unproductive shrubland into a Mediterranean dehesa. J. Environ. Manage. 82, 446–456.

Trubat, R., Cortina, J., Vilagrosa, A., 2006. Plant morphology and root hydraulics are altered by nutrient deficiency in *Pistacia lentiscus* (L.). Trees – Struct. Funct. 20, 334–339.

Valdecantos, A., Vilagrosa, A., Seva, J.P., Cortina, J., Vallejo, V.R., Bellot, J., 1996. Mycorhhization et application de compost urbain pour l'amélioration de la survie et de la croissance des semis de *Pinus halepensis* en milieu semi-aride. Options Mediterraneennes 20, 87–104.

Valdecantos, A., Fuentes, D., Cortina, J., 2004. Utilización de biosólidos en la restauración forestal. In: Vallejo, V.R., Alloza, J.A. (Eds.), Avances en el estudio de la gestión del monte Mediterráneo. Fundación CEAM. Valencia, Spain, pp. 313–344.

Valdecantos, A., Cortina, J., Vallejo, V.R., 2006. Nutrient status and field performance of tree seedlings planted in Mediterranean degraded areas. Ann. For. Sci. 63, 249–256.

Valdecantos, A., Baeza, M.J., Vallejo, V.R., 2009. Vegetation management for promoting ecosystem resilience in fire-prone Mediterranean shrublands. Restor. Ecol. 17, 414–421.

Valentini, R., Scarascia Mugnozza, G.E., Ehleringer, J.R., 1992. Hydrogen and carbon isotope ratios of selected species of a Mediterranean macchia ecosystem. Funct. Ecol. 6, 7–63.

Vallejo, R., Aronson, J., Pausas, J.G., Cortina, J., 2006. Restoration of Mediterranean woodlands. In: van Andel, J., Aronson, J. (Eds.), Restoration Ecology: The New Frontier. Blackwell Science, pp. 193–207.

Vega-García, C., Chuvieco, E., 2006. Applying local measures of spatial heterogeneity to Landsat-TM images for predicting wildfire occurrence in Mediterranean landscapes. Landsc. Ecol. 21, 595–605.

Villar-Salvador, P., Planelles, R., Enriquez, E., Peñuelas Rubira, J., 2004. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. Forest Ecol. Manag. 196, 257–266.

Villar-Salvador, P., Puértolas, J., Peñuelas, J.L., Planelles, R., 2005. Effect of nitrogen fertilization in the nursery on the drought and frost resistance of Mediterranean forest species. Invest. Agrar.: Sist. Recur. For. 14, 408–418.

Wang, H., Magesan, G.N., Clinton, P.W., Lavery, J.M., 2005. Using natural ^{15}N abundances to trace the fate of waste-derived nitrogen in forest ecosystems: New Zealand case studies. Isot. Environ. Health Stud. 41, 31–38.

Wang, H., Magesan, G., Kimberley, M., Payn, T., Wilks, P., Fisher, C., 2004. Environmental and nutritional responses of a *Pinus radiata* plantation to biosolids application. Plant Soil 267, 255–262.

Weetman, G.F., 1989. Graphical vector analysis technique for testing stand nutritional status. In: Dyck, W.J., Mees, C.A. (Eds.), Research Strategies for Long-Term Site Productivity. Ministry of Forestry of New Zealand, pp. 93–109.

Zasoski, R.J., Cole, D.W., Bledsoe, C.S., 1983. Municipal sewage sludge use in forests of the Pacific Northwest, U.S.A.: growth responses. Waste Manage Res. 1, 103–104.