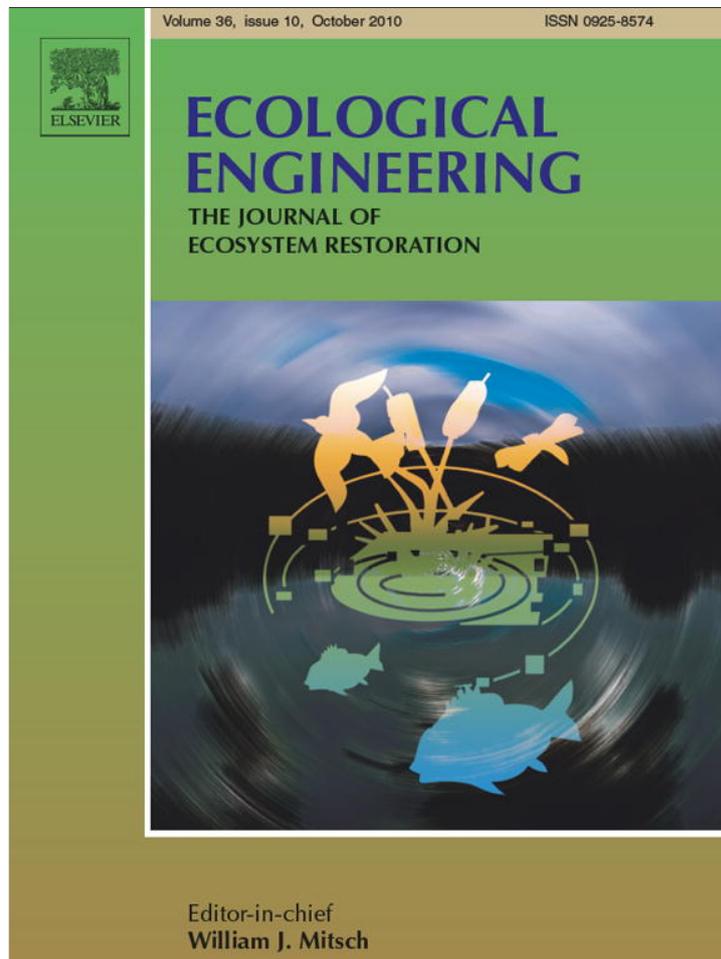


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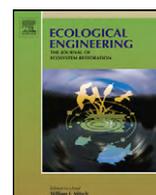
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Fine-tuning of sewage sludge application to promote the establishment of *Pinus halepensis* seedlings

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ABSTRACT

Planting woody vegetation is frequently a first step towards the restoration of degraded drylands. Seedling establishment on unfertile soils may be favoured by applying organic amendments such as biosolids. But the outcome of such a practice is strongly dependent on the type of amendment and the application rate used. High application rates may have deleterious effects on plant performance and compromise plantation success. Thus amendment type and dose should be carefully selected to optimise benefits and minimize risks. In this study, we evaluated the effect of two organic amendments (composted and air-dried sewage sludge) applied at 5 doses (0, 15, 30, 45 and 60 Mg ha⁻¹) on soil properties and on the performance of 1-year-old *Pinus halepensis* seedlings planted in a dry Mediterranean degraded area. Soil organic matter, electrical conductivity and nutrient availability increased with the application rate, but the magnitude of this increase depended on the type of amendment and the time. Organic amendments improved N and P status and promoted seedling growth. Nevertheless, at the higher application rates they showed a negative impact on seedling survival 1 and 3 years after application. Drought effects intensified by root competition with extant vegetation and reduced water availability within the planting hole were the main causes attributed to the higher mortality. Low to moderate doses showed the best combination of seedling survival and growth and can thus be recommended to promote the establishment of *P. halepensis* in dry Mediterranean areas.

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1. Introduction

The wastewater depuration process generates a by-product rich in organic matter and nutrients (Henry and Cole, 1997; Marx et al., 1995). Its reutilization has been recommended as the best practicable environmental option (Hall, 1999). Moreover, as the generation rate of biosolids has steadily increased worldwide, the identification of new disposal routes can be of interest to wastewater companies and managers (USEPA, 1999). In drylands, use of organic amendments may improve soil conditions and promote ecosystem recovery (Querejeta et al., 2000; Tarrasón et al., 2007).

Dryland forests and rangelands often show low plant cover due to intense historical use (Yaalon, 1997). In these areas, human intervention may be needed to deter further degradation and foster succession. The reintroduction of key woody species is commonly

the first step towards their restoration (Vallejo et al., 2000), but seedling survival and growth is often poor because of adverse climate and soil conditions (Pausas et al., 2004). Nutrient limitation affects seedling ability to root (Oliet et al., 2009; Trubat et al., 2008) and may compromise its performance (Valdecantos et al., 2006). Nutrient-rich organic amendments can improve soil physical and chemical properties, increasing water holding capacity and nutrient availability, and promoting seedling establishment (Pascual et al., 1999; Querejeta et al., 1998; Roldan et al., 1996).

Protocols for the application of nutrient-rich organic amendments for dryland restoration differ from those used in agriculture and land reclamation. In restoration, the use of organic amendments may be justified as a kick-off treatment to improve early growth in planted seedlings, and should be restricted in time (e.g., one single application before planting) and space (application not widespread, but confined to planting holes or lines). In this way, most of the restoration actions in drylands of the Mediterranean Basin include the introduction of key species in planting holes, but there is little information on the application of increasing doses and different types of biosolids taking into account these constraints (Caravaca et al., 2003; Larchevêque et al., 2006, 2008; Pascual et al., 1999). Unintended effects of this practice, such as the

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disturbance of soil surface and extant vegetation, and the establishment of ruderal and alien plant species, could be minimised by optimizing the type and doses, as well as the techniques used in their application. But the net effect of this practice is highly dependent on other biotic and abiotic factors such as soil characteristics, climatic conditions and operational constraints (Jacobs and Timmer, 2005; Pascual et al., 1999; Zagas et al., 2000). Reduction of water availability (Brockway, 1983), unbalanced nutrition (Harrison et al., 1996), and competition with standing vegetation (Berry, 1979; Cogliastro et al., 2001), have been identified as probable sources of stress for seedlings receiving organic amendments. Thus, detailed information on the interplay between organic amendments, climate, soils and introduced plants is needed to maximize the benefits and minimize the risks of their application.

The objectives of this study are (1) to identify the optimum application rates of two types of organic amendments (composted and air-dried sewage sludge) for the establishment of *Pinus halepensis* Mill. (Aleppo pine) seedlings in degraded Mediterranean dry areas and (2) to explore the main factors affecting seedling response to organic amendments. Our hypothesis is that the doses employed in agroforestry systems (Cheng et al., 2007; Dolgen et al., 2004; USEPA, 1995) may have deleterious effects on seedling performance in forest restoration, given the constraints on the use of organic amendments discussed above.

2. Materials and methods

2.1. Study site and experimental design

The study area is located in Enguera (Valencia, E Spain) under dry sub-humid Mediterranean climate (mean annual temperature 12.7 °C, mean annual precipitation 479 mm). The site has burned twice in the last 25 years (the latest in 1999), resulting in a shrubland dominated by *Quercus coccifera*, *Cistus* sp. and *Rosmarinus officinalis*, and a few isolated trees of *Pinus pinaster* and *P. halepensis* surviving or regenerating from the previous forest. Soils are sandy loam (62% sand, 17% clay and 21% silt) and alkaline (pH = 7.95 ± 0.05) with high carbonate content (62 ± 2%) developed from limestone and marl (*Cambisols*). In February 2003, a backhoe excavator dug 40 cm × 40 cm × 40 cm holes along 0.5 ha of gentle slopes (0–15%). The mean soil depth after soil preparation across the study site was 33.5 ± 1.6 cm (mean and standard error of 13 profiles in planting holes).

Composted sewage sludge (hereafter compost) from a composting plant in Aspe (Alicante) and air-dried anaerobically digested sewage sludge (hereafter dry sludge) (Table 1) from a domestic wastewater treatment plant in Ayora (Valencia) were applied and mixed with the soil in the planting holes at four rates corresponding to 15, 30, 45 and 60 Mg dry weight ha⁻¹ (243–945 g d.w. and 240–960 g d.w. of dry sludge and compost per planting hole, respectively). Additional unamended planting holes acted as controls. Treatments were randomly distributed along the plot. At these application rates, soil organic matter at the 0–30 cm depth increased in a range of 0.15–0.70% depending on the specific treatment.

Soon after site preparation (1 week later), we planted thirty 1-year-old *P. halepensis* seedlings per amendment type and dose. *P. halepensis* is one of the most important tree species in the Mediterranean Basin, covering more than 25,000 km² and dominating forest formations in semiarid and dry sub-humid areas (Quezel, 2000). Seedlings were produced in a public forest nursery (La Hunde, Valencia), 10 km from the study area. They were grown in 350 cm³ forest containers filled with peat and cocopeat

Table 1

Physico-chemical properties of the organic amendments used in this study.

	Compost ^{a,b}	Dry sludge ^b
Moisture content (%)	40	10
Total organic matter (%)	62.6	38.3
Organic C (%)	29.1	22.2
pH (1:10)	6.3	6.7
EC (1:10, dSm ⁻¹)	5.54	4.58
C/N	10.0	4.3
Total N (%)	2.98	3.50
Total P (%)	0.67	1.57
K (%)	0.49	0.21
Ca (%)	3.98	nd
Mg (%)	0.49	nd
Na (%)	0.76	nd
Fe (ppm)	32.0	2.4
Mn (ppm)	213	70
Cd (ppm)	3	<2
Cr (ppm)	43	25
Cu (ppm)	169	131
Pb (ppm)	231	54
Zn (ppm)	504	480
Ni (ppm)	17	16
Hg (ppm)	1	<0.75

^a Straw and sawdust as bulking agents.

^b Total heavy metal concentration

(1:1, v/v). No pre- and post-planting treatment was performed in the plot.

2.2. Plant performance

Seedling survival and growth (shoot height and basal diameter) were measured 2, 5, 7, 10, 20, 27 and 45 months after planting. Pine basal area per hectare was estimated by summing the basal areas of each individual seedling and assuming an initial planting density of 800 seedlings ha⁻¹. In November 2006, we harvested current year needles from 5 seedlings per biosolid type and dose (except for the higher doses of compost where $N=3$). Needles were digested in a heating block at 250 °C with a mixture of concentrated sulphuric acid and 30% hydrogen peroxide (1:1, v:v) (Jones and Case, 1990). Total N was measured using semi-micro Kjeldahl distillation (Tecator Kjeltac Auto 1030 Analyzer, Hogana, Sweden). Phosphorus, K, Cu and Zn were analysed by ICP-OES (Perkin Elmer Optima 4300 Inductively Coupled Plasma-Optical Emission Spectrometry, Perkin Elmer Corp., Norwalk, CT, USA).

2.3. Soil properties

We monitored soil water content during the first year after planting by using Time Domain Reflectometry (TDR Tektronik 1502C Cable Tester), with one vertical set of probes (0–20 cm depth), twice during summer drought (July 25th and August 4th) and after two late summer storms (August 23rd and September 11th) in 10 planting holes per biosolid type and dose. We calculated gravimetric water content using the Topp equation (Topp and Davis, 1985) and the bulk soil density values. We used a 162 cm³ volumetric bore auger to estimate bulk density of the fine earth (<2 mm diameter). Soil water retention curves (drying path) were obtained for soils amended with dry sludge using a dewpoint hygrometer (WP4, Decagon Devices, Inc., Pullman, WA). Parameters for the mathematical representation of the water retention curve, using a power law model, are shown in Table 3.

Ten soil samples (0–25 cm) per treatment (except for 45 Mg ha⁻¹) were taken in July 2003 to measure soil organic matter (OM), electrical conductivity (EC) and pH. Soil EC was determined from extracts of a saturated soil-paste (Rhoades, 1982) and measured by a conductivity meter (Crison C-525, Crison Instruments,

Table 2

F values and significance of the analysis of variance on soil properties after one (2003) and three (2006) summers in the field to evaluate the effect of amendment type (AT) and dose (D).

	OM		EC		PMN		N		P	
	2003	2006	2003	2006	2003	2006	2003	2006	2003	2006
F_D	9.5***	0.9	30.5***	0.53	22.7***	nd	nd	1.1	nd	3.5*
F_{AT}	8.1**	2.7	17.8***	1.59	14.4***	nd	nd	0.5	nd	7.7**
$F_{AT \times D}$	0.3	1.6	8.2***	1.94	4.3*	nd	nd	4.3*	nd	0.3
$F_{compost}$	8.6***	1.7	7.8***	2.96*	7.8***	nd	nd	4.6**	nd	8.9***
F_{sludge}	5.4**	2.1	38.9***	2.51	17.6***	nd	nd	1.9	nd	1.8

* Significant at $p < 0.050$.

** Significant at $p < 0.010$.

*** Significant at $p < 0.001$.

Barcelona, Spain). Soil organic matter content was determined by the Walkley and Black method (Nelson and Sommers, 1996). After the first autumn rains, we collected new soil samples and measured NH_4^+ and potentially mineralizable nitrogen (PMN). We estimated PMN on samples subjected to anaerobic conditions for 1 week (Keeney, 1982) as the increase in NH_4^+ concentration during the incubation (difference between final and initial concentration). N-NH_4^+ was determined by the indophenol-blue method (Keeney and Nelson, 1982). Potentially mineralizable N estimated in this way has been related to plant response to fertilization (Powers, 1980).

In November 2006, a new set of soil samples was collected from the planting holes (10 replicated planting holes per amendment type and dose, except for 45 Mg ha^{-1}). We estimate bulk density (as described above) and we determined the pH, electrical conductivity, organic matter (as described above), total nitrogen (TruSpec CN analyser), and available phosphorous (Olsen and Sommers, 1982). To estimate aggregate stability, we gently sieved the samples to obtain the soil fraction between 2 mm and 0.2 mm and used a probe-type sonifier (Sonifier I Ultrasonic Cell Disruptor Model 250; Branson Ultrasonic SA, USA) to calculate the percentage of aggregates larger than 0.2 mm remaining after exposure to 10 W of power for 10 s in an aqueous medium (modified from Cerdà, 1996).

Finally, we determined potential belowground interference from extant vegetation by determining fine root density in 10 planting holes per biosolid type and dose. Soil samples from the 0 to 20 cm depth were collected during the second summer (2004) after planting and at the end of the experiment (November 2006).

2.4. Statistical analysis

Soil properties, seedling growth and nutritional status were first analyzed separately for each type of amendment using one-way analysis of variance with Dose (D) as a fixed factor. Then, we used a two-way univariate analysis of variance with type of amendment (TA) and Dose as fixed factors. Control seedlings were excluded from the latter analyses to avoid missing cells. Pairwise comparisons were performed with the Student–Newman–Keuls test (SNK) at the 0.05 significance level when ANOVA revealed significant treatment effects. Data transformations were carried out when necessary to ensure the validity of the assumptions of normality, linearity, and homoscedasticity.

We evaluated the effects of amendment type and dose on first year and third year seedling survival by non-parametric log-rank test (Fox, 1993). The same type of analyses was used to evaluate the effect of application dose on seedling survival separately for each amendment type.

We used linear and quadratic regressions to assess the relationship between soil properties and seedling survival and Pearson's r to assess the degree of covariation between soil variables at the end

of the experiment. All statistical analyses were performed using the SPSS v.13.0 statistical package (SPSS Inc., Chicago, USA).

3. Results

3.1. Effect of organic amendments on soil

Organic matter content was higher in soils amended with compost than in those receiving dry sludge after the first summer. Soil OM increased with dose (Table 2 and Fig. 1), but only the highest doses differed from the rest. The results were similar when unamended soils were included in the analyses. The trend was similar 3 years after planting, but the differences were no longer significant.

During the first summer, soil EC was significantly higher when soils were amended with dry sludge as compared to compost. The application of 60 Mg ha^{-1} of organic amendments increased EC above the levels measured at 15 and 30 Mg ha^{-1} application rates. Soils receiving dry sludge at any dose showed higher EC than control soils, whereas only soils receiving 60 Mg ha^{-1} of compost differed from unamended soils. Three years after planting, differences in soil EC between soils amended with dry sludge were not significant, whereas soils that received 30 and 60 Mg ha^{-1} of compost still had higher EC than control soils. Soil pH ranged between 7.72 and 7.95 one year after planting and 7.65–7.96 three years later. Organic amendments had no significant effect on this variable (data not shown).

The potentially mineralizable N of soils amended with dry sludge was higher than PMN in compost-amended soils after the first summer. There was a substantial increase in PMN when amendments were applied at 60 Mg ha^{-1} rate, but we found no significant effect of the amendments when applied at lower doses. Average soil EC and PMN were strongly and positively correlated ($R^2 = 0.995$, $p < 0.001$, $N = 7$).

Three years after planting, soil N concentration showed no differences between type of amendment or doses but there was a significant interaction. Within each type of amendment, the N concentration in soils receiving 60 Mg ha^{-1} of compost was higher than that of soils amended with the lowest doses (Fig. 2).

Soil phosphorus availability 3 years after planting was higher when the amendment was applied as compost as compared to dry sludge (Fig. 2). Dose had a significant effect on soil available P when both amendment types were analysed together and when compost was analyzed separately, but not when soils received dry sludge.

Dose, but not amendment type, had a negative effect on volumetric soil moisture content of intermediate doses after a rainfall event as compared with the highest dose (Table 3). The characteristic moisture curve for soils amended with dry sludge also showed a gradual decrease in water availability with increasing doses. Three years after biosolid application no significant differences between treatments were found with respect to aggregate stabil-

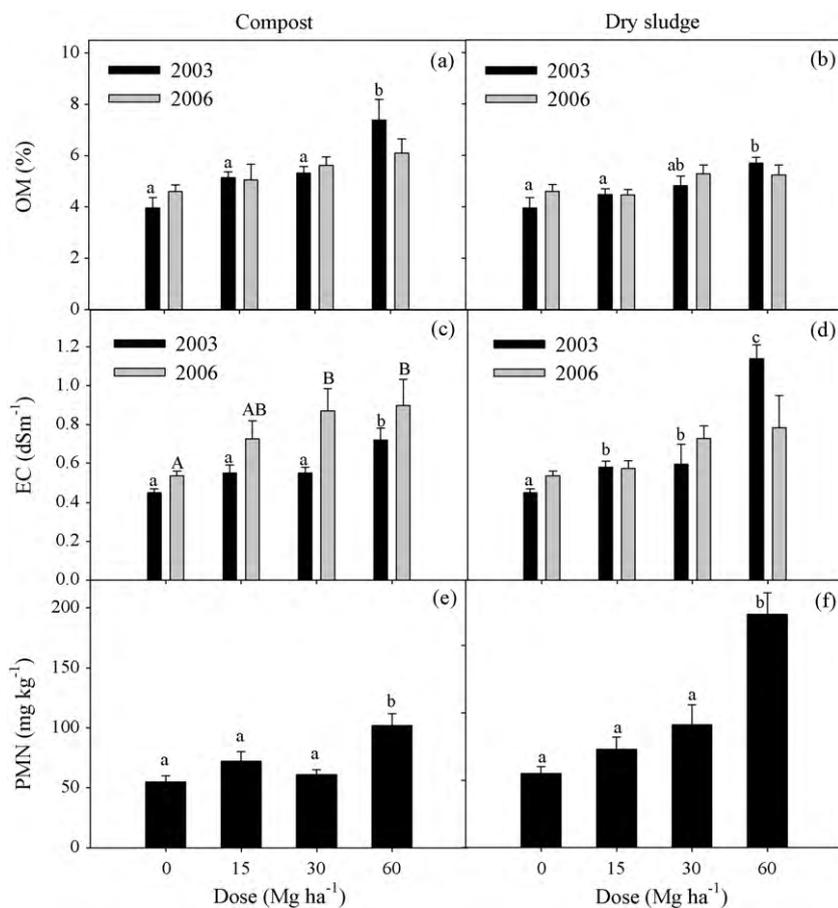


Fig. 1. Soil organic matter (OM), electrical conductivity (EC) and potentially mineralizable nitrogen (PMN) in the planting holes amended with compost (a, c, e) and dry sludge (b, d, f) at different application rates during the first summer after planting, and 3 years later (except for PMN). Data represent means \pm SE ($N=9-10$ soil samples). Results of a post hoc SNK test ($p < 0.05$) for each amendment type are indicated by different letters.

ity, which ranged between $50 \pm 4\%$ and $57 \pm 5\%$, or bulk density, between $0.8 \pm 0.1 \text{ g cm}^{-3}$ and $0.9 \pm 0.1 \text{ g cm}^{-3}$. Nevertheless, the organic matter content in soil continued to be positively correlated with the soil N concentration for soils receiving both amendment types (Pearson's $r = 0.823$, $p < 0.001$ for compost and $r = 0.682$, $p < 0.001$ for dry sludge), and EC in soil ($r = 0.701$, $p < 0.001$ and $r = 0.515$, $p < 0.001$). A negative relationship was found only with bulk density ($r = -0.699$, $p < 0.001$) in compost-amended soils.

3.2. Seedling nutrient status

Three years after planting, we found no effect of amendment type and dose on N, P or K concentration in needles (Table 4). When both types of amendments were analysed separately, the application of 30 Mg ha^{-1} of dry sludge significantly increased needle N concentration as compared with control seedlings. In contrast, P concentration was higher for any dose of amendment as compared with control seedlings. At said point of time, needle concentrations of K, Cu and Zn showed no differences with those found in control seedlings.

3.3. Seedling survival

Seedling survival ranged from 79–30% to 52–10% at the end of the first and third summer, respectively (Fig. 3). Amendment dose, but not amendment type, had a significant effect on this variable on both sampling dates ($\chi^2 = 18.89$, $p < 0.001$ and $\chi^2 = 18.40$, $p < 0.001$, for the first and third year, respectively). Thus, at the end of the first

summer in the field, seedling survival was reduced by 30% and 50% when amendments were applied at 45 and 60 Mg ha^{-1} in compost and dry sludge, respectively ($\chi^2 = 7.16$, $p = 0.007$ and $\chi^2 = 18.57$, $p < 0.001$) as compared with the 30 Mg ha^{-1} treatment which maintained higher survival rates. At this date, seedling survival was not related to the application rate but negatively related to the soil EC ($R^2 = 0.785$, $p = 0.003$, $N = 7$). After 3 years, the effect of the compost and dry sludge application rate on survival was still significant ($\chi^2 = 5.85$, $p = 0.016$ and $\chi^2 = 13.89$, $p = 0.001$, respectively) and it was negatively related by linear and quadratic functions to the doses applied. In this case no relationship was found between survival and soil EC.

3.4. Seedling growth

We found no effect of amendment type and application rate on stem height when both amendments were analysed together ($F_{TA} = 0.17$, $p = 0.68$ and $F_D = 1.56$, $p = 0.21$). Similar results were obtained for root collar diameter (data not shown). In contrast, stem height was enhanced by organic amendments applied at any dose in the first year after planting when both amendment types were analysed separately ($F = 4.2$, $p = 0.004$ and $F = 5.5$, $p = 0.001$ for compost and dry sludge, respectively) (Fig. 4). After the second summer, the lowest doses of dry sludge maintained their positive effect on seedling growth, as did the compost applied at 30 Mg ha^{-1} ($F = 3.1$, $p = 0.021$ and $F = 4.19$, $p = 0.008$ respectively). Higher doses of compost and dry sludge had either no effect or a negative effect on seedling growth. The strong droughts of 2005 and 2006 resulted

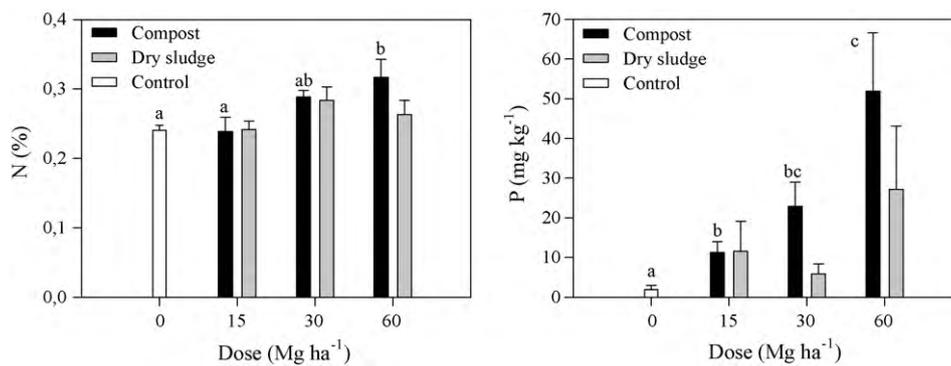


Fig. 2. Total N (left) and available P (right) in the soil of the planting holes amended with compost and dry sludge at different application rates 3 years after planting. Data represent means \pm SE ($N=9-10$). When significant differences were detected by one-way ANOVA, the results of an SNK test ($p < 0.05$) are indicated by letters within each type of amendment.

Table 3

Soil moisture in the planting holes amended with compost, dry sludge or unamended during the peak of summer drought and after a late summer storm (August 4th and September 11th). The characteristic moisture curve was estimated only for dry sludge and control treatments. The resulting ψ_s values are given. When significant differences were detected by one-way ANOVA, the results of an SNK test ($p < 0.05$) are indicated by letters within each type of amendment.

Dose (Mg ha ⁻¹)	Soil moisture (%) (during drought)		ψ_s (MPa) ^a	Soil moisture (%) (after rain)		ψ_s (MPa)	a^a	b	R^2
	Compost	Dry sludge		Compost	Dry sludge				
0	5.1 \pm 0.2		-1.24 \pm 0.17a	22.6 \pm 0.4a		-0.02 \pm 0.00a	-109	-2.7	0.991***
15	5.3 \pm 0.2	5.6 \pm 0.4	-2.42 \pm 0.33b	20.9 \pm 0.5b	21.0 \pm 0.9	-0.09 \pm 0.01b	-136	-2.44	0.998***
30	4.9 \pm 0.2	5.2 \pm 0.3	-4.61 \pm 0.64c	20.3 \pm 0.5b	20.5 \pm 0.5	-0.11 \pm 0.01c	-310	-2.63	0.990***
60	5.3 \pm 0.2	5.5 \pm 0.4	-6.39 \pm 0.95c	22.9 \pm 0.5a	22.9 \pm 0.7	-0.18 \pm 0.02d	-337	-2.41	0.990***
One-way F	0.59	0.67	23.0***	6.03***	2.84	70.1***			
Two ways									
F_D	0.78			6.71**					
F_{AT}	3.70			0.08					
$F_{D \times AT}$	0.006			0.08					

^a A power law model was used to fit the relationship between ψ and soil moisture.

* $p < 0.050$.

** $p < 0.010$.

*** $p < 0.001$.

in the attenuation of seedling growth and a substantial decrease in seedling survival. As a result, basal area decreased during the last 2 years of the experiment, especially in the compost treatments with reductions to 1.21 and 1.41 m² ha⁻¹ in the 15 and 30 Mg ha⁻¹ treatments, respectively. In contrast, the equivalent doses of dry sludge reached 3.75 and 2.72 m² ha⁻¹, respectively (Fig. 4). The highest dose resulted in similar basal area values

for both amendment types, and remained lower than the control treatment.

3.5. Colonization of the planting hole by extant vegetation

Root density in the planting holes during the second summer was enhanced with increasing amendment dose ($F_D = 4.19$,

Table 4

Needle nutrient status of *Pinus halepensis* seedlings 40 months after planting. Mean and standard error of $N=4-5$ seedlings per treatment and results of a one-way ANOVA (with D as the only factor) and a two-way ANOVA (with TA and D as factors) are also included. When significant differences were detected by one-way ANOVA, the results of an SNK test ($p < 0.05$) are indicated by letters within each type of amendment.

	N (%)		P (%)		K (%)		Cu (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
	Compost	Dry sludge	Compost	Dry sludge	Compost	Dry sludge	Compost	Dry sludge	Compost	Dry sludge
0	1.12 \pm 0.12a		0.09 \pm 0.01a		0.28 \pm 0.04		3.5 \pm 0.7		24.4 \pm 3.9	
15	1.40 \pm 0.07	1.51 \pm 0.11ab	0.15 \pm 0.01b	0.18 \pm 0.01b	0.30 \pm 0.02	0.27 \pm 0.01	3.6 \pm 0.2	3.6 \pm 0.2	33.9 \pm 2.2	32.6 \pm 2.7
30	1.64 \pm 0.23	1.81 \pm 0.08b	0.16 \pm 0.02b	0.18 \pm 0.04b	0.31 \pm 0.02	0.30 \pm 0.03	3.7 \pm 0.4	4.1 \pm 0.6	27.8 \pm 4.9	35.6 \pm 3.9
60	1.68 \pm 0.14	1.55 \pm 0.31ab	0.16 \pm 0.03b	0.19 \pm 0.03b	0.26 \pm 0.06	0.28 \pm 0.03	3.6 \pm 0.1	3.0 \pm 0.4	31.2 \pm 6.6	25.1 \pm 0.4
One-way F	3.17	3.49*	3.98*	16.4**	0.4	0.2	0.1	0.6	1.3	2.5
Two ways										
F_D	0.31		0.1		0.7		1.3		1.6	
F_{AT}	0.15		3.8		0.04		0.1		0.01	
$F_{D \times AT}$	1.85		0.2		0.6		1.3		1.1	

* $p < 0.050$.

** $p < 0.010$.

*** $p < 0.001$.

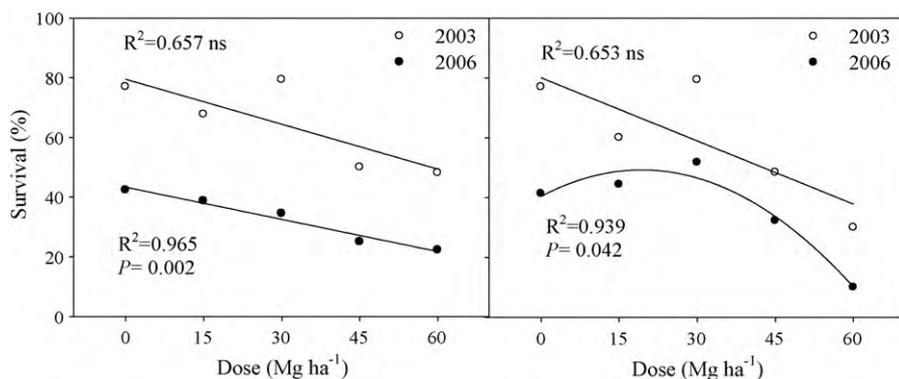


Fig. 3. Changes in the survival of *Pinus halepensis* seedlings at increasing doses of compost (left) and dry sludge (right). Data correspond to data recorded seven months (open circles) and 45 months after planting (closed circles), and were adjusted to linear and quadratic equations.

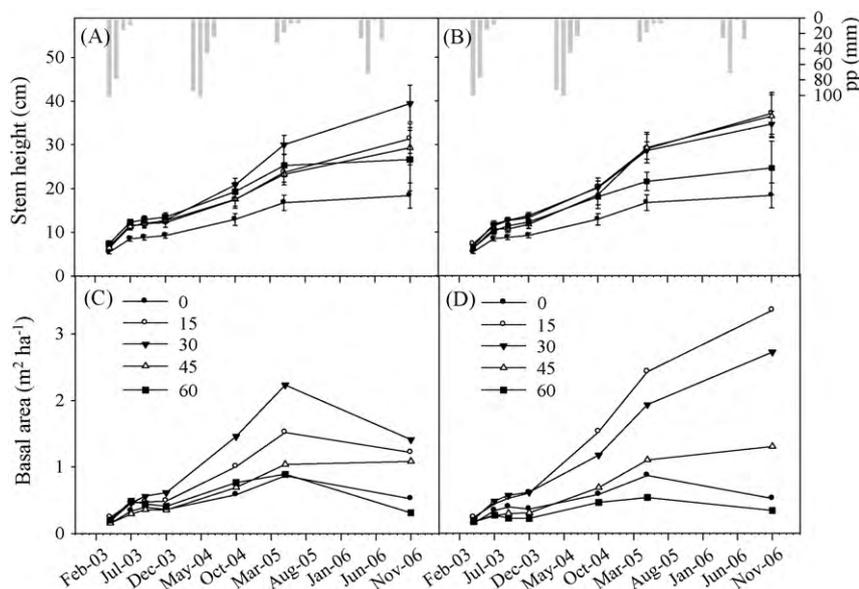


Fig. 4. Seedling average stem height (a, b) and total basal area (c, d) of *P. halepensis* seedlings amended with increasing doses of compost (a, c) and dry sludge (b, d) applied in the planting hole. Stem height data represent means \pm SE. The distribution of monthly precipitation between April and July during the 2003–2006 growing seasons is showed in the upper graphs.

$p=0.021$) but no differences were found between amendment type ($F_{AT}=0.10$, $p=0.753$). Soils receiving dry sludge at any dose showed higher root density than control soils, whereas only soils receiving 60 Mg ha^{-1} of compost differed from unamended soils. Application of 60 Mg ha^{-1} of dry sludge showed the highest root density ($7.6 \pm 1.3 \text{ mg cm}^{-3}$) while control holes showed the lowest ($1.6 \pm 0.2 \text{ mg cm}^{-3}$). After 3 years only marginal differences remained in soils amended with dry sludge between control and 60 Mg ha^{-1} treatments ($F=2.49$, $p=0.070$). Soil EC and root density in the planting holes had a significant and positive relationship in the soils that received dry sludge ($R^2=0.015$, $p=0.77$ and $R^2=0.887$, $p<0.001$ for compost and dry sludge, respectively).

4. Discussion

4.1. Effect of organic amendments on soil

Only the highest dose of organic amendments increased soil OM significantly. This effect vanished 3 years later. These results agree with the ones obtained by Larchevêque et al. (2006) with composted sewage sludge under Mediterranean field conditions. The differences in OM mineralization kinetics between compost and

dry sludge treated soils were supported by the differences in PMN and EC observed after the first summer in the amended soils. Treatments with low application rates showed no differences with the control soil, suggesting that at low application rates most of the labile organic compounds may have been oxidised below detection levels 28 weeks after planting. The organic fractions of less stabilized organic amendments mineralized faster than those previously composted (Hernández et al., 2002; Pascual et al., 1999), which caused an initial increase in nutrient availability and soil EC in the dry sludge amended soils. Likewise, despite the higher amount of P ($2\text{--}6 \text{ g P seedling}^{-1}$ and $4\text{--}15 \text{ g P seedling}^{-1}$ for compost and dry sludge, respectively) and N ($7\text{--}29 \text{ g N seedling}^{-1}$ and $9\text{--}33 \text{ g N seedling}^{-1}$ for compost and dry sludge, respectively) supplied to the soil with the dry sludge, the compost-treated soils showed a higher availability of both elements 3 years after the application. At this date, the significant relationship with soil N and EC also suggests that the recalcitrant organic matter added with the biosolids was still in a phase of slow mineralization (Pascual et al., 1999), especially in the compost application.

The relationship between OM concentration and other soil properties (total N and EC) at the end of the experiment suggests that, in the medium term, biochemical properties were more sensi-

tive than physical properties to the organic amendments. At this date, the amendments slightly affected soil aggregate stability and bulk density. It has been demonstrated that increases in soil OM enhance water holding capacity (Querejeta et al., 2000). However, in our study, increasing the biosolid application rate had no effect on soil moisture in the planting holes, especially during the first drought period after plantation. Amendment concentration (i.e. dose and degree of incorporation), amendment type, and soil and climate properties control the effect of organic amendments on soil physical improvement (Caravaca et al., 2003; Querejeta et al., 2000), which is commonly reported to occur when amendments are applied at higher doses than those used in our study (Bastida et al., 2007; Cox et al., 2001; Navas et al., 1998; Sort and Alcañiz, 1996). Moreover, the increase in soil fertility in amended soils promoted root colonization of planted *P. halepensis* and extant vegetation, probably increasing transpiration losses. Belowground interference in drylands can be much stronger than aboveground (Pérez-Devesa et al., 2008) and, indeed, Mediterranean vegetation strongly responds to localized nutrient enrichment by increasing root proliferation (Valdecantos et al., 2006).

4.2. Effects of organic amendments on seedling survival

The survival of *P. halepensis* was negatively related to soil salinity during the first summer. Phytotoxicity was unlikely because common symptoms, such as necrotic needles and abnormal needle colours, were not evident and seedling morphology was not negatively affected by the organic amendments during the growing period. It must be noted that EC values correspond to discrete measurements and may not reflect transient peaks in salinity. Fertilization under warm, dry weather has often been associated with higher seedling mortality, due to aggravated seedling stress, mainly as a result of the osmotic effect of salts (Rose and Ketchum, 2002; van den Driessche et al., 2003) or weed competition (Ramsey et al., 2003). Soluble salts released during OM mineralization may accumulate in the rizosphere (Jacobs and Timmer, 2005; Jacobs et al., 2004) particularly as leaching is absent and evaporative demand high during long periods of time (Bastida et al., 2007). Indeed, site preparation by digging holes favoured the physical microenvironment of the planting spots for spontaneous root colonization. Therefore, the synergistic effect of soil drying by water uptake from roots of either pine seedling and natural vegetation and the increase in soluble salts after the application of 60 Mg ha⁻¹ of dry sludge during the first summer drastically decreased soil water potential and seedling survival. During the first year after planting, the root plug is mostly confined to the soil surface and thus it may be particularly sensitive to planting hole conditions. These conditions may extend in time in areas where root progress is hampered by the proximity of impenetrable bedrocks and soil horizons (Ganatsas and Spanos, 2005). Increased nutrient availability in the surface soil and relatively benign years after planting may also have favoured root confinement in the upper soil horizons. This may explain the unusual mortality rates recorded after the second summer when the April–July accumulated rainfall in 2005 and 2006 was much lower than the first 2 years and below the historical records (213, 286, 63 and 126 mm in 2003, 2004, 2005 and 2006, respectively as compared with the 133 mm of the historical series). It has been proposed that when rainfall falls below the historical records the competition between natural and planted vegetation may release a peak in mortality percentages (Maestre et al., 2002). In fact, Navarro Cerrillo et al. (2005) reported an increase of seedling survival of holm oak after weed management probably due to both higher resource availability and improved seedling microenvironment. In addition, we observed that, at the end of the second dry year, salt release in the dry sludge treatments seemed to be exhausted and

mortality stabilised while compost treatments above 15 Mg ha⁻¹ maintained higher EC values than control soils, contributing to the drop in survival in the 30 Mg ha⁻¹ of compost. Probably the higher seedling size in this treatment indicated a higher water demand, which could enhance seedling stress when the dry period began (winter 2004–2005). Similar effects were obtained by Roldan et al. (1996) after an unusually dry spring in plots afforested with *P. halepensis* and amended with USR in manual planting holes.

4.3. Effect of organic amendments on seedling nutrient status and growth

Organic amendments commonly produce positive effects on *P. halepensis* growth (Querejeta et al., 2000; Valdecantos, 2001), especially in relation to increases in available soil P (Roldan et al., 1996; Valdecantos et al., 2006) and, to a lesser extent, increases in soil N (Fuentes et al., 2007). Soil N and available P in unamended soil of the area show low values but similar to those found in the literature (Sardans et al., 2008; Zornoza et al., 2007) which may be sufficient to sustain the establishment of *P. halepensis* seedlings (Valdecantos et al., 2006). Increased root colonization of amended soils, poor nutrient status of control plants and low soil P availability (Larchevêque et al., 2006; Valdecantos et al., 2006) also suggested that, in addition to water availability, nutrients were limiting plant performance on the study site.

During the first 2 years after planting the lowest dose of dry sludge had similar effects on seedling growth than 30 Mg ha⁻¹ of compost, reinforcing the different effects on soil shown by the amendments concerning to the lower maturity of dry sludge and higher N and P initial concentration. Higher needle N concentrations in the present study were positively related to growth, but showed negative or no relationship with survival, indicating that the increase in N supply rate did not ameliorate plant stress or was outbalanced by adverse osmotic effects. Needle P concentration did not differ between doses; thus, the lowest dose of biosolid (15 Mg ha⁻¹) was enough to optimize P status in seedlings. However, we found a high P needle concentration in treated seedlings after two consecutive dry growing periods, suggesting a high temporal P availability in soil probably due to a combined effect of OM and moisture regime on P-insolubilisation in calcareous soils, as described by Braschi et al. (2003), with a strong negative relationship between P availability and accumulated rainfall after fertilization.

5. Conclusion

The use of organic refuses in the restoration of degraded areas proved effective in increasing seedling growth and basal area in planted *P. halepensis* seedlings, but not in increasing their survival. Considerations to take into account when biosolids are employed in dry or semiarid environments are different from those needed for less stressful sites. Introduced seedlings seem to be sensitive to increases in site-specific stress factors such as, in our conditions, the occurrence of intense drought periods together with the increment in soil EC and probably soil physical constraints. This implies a higher risk for the seedlings when the soil dries out, especially if they have not been able to colonize the soil beyond the planting hole. The use of amendments can intensify drought effects through root competition with extant vegetation and reduced water availability within the planting hole with doses higher than 30 Mg ha⁻¹. Nevertheless, we found that the use of composted amendments, with their gradual release of salts and nutrients into the soil, has a clear advantage in restoration actions restricted to a single initial application because their effects last longer than non-composted

ones; this application must, however, be accompanied not only by a careful selection of adequate doses but also by site preparation to prevent mortality episodes after intense drought periods.

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References

- Bastida, F., Moreno, J.L., García, C., Hernández, T., 2007. Addition of urban waste to semiarid degraded soil: long-term effect. *Pedosphere* 17, 557–567.
- Berry, C.R., 1979. Slit application of fertilizer tablets and sewage sludge improve initial growth of loblolly pine seedlings in the Tennessee Copper Basin. *Reclam. Rev.* 2, 33–38.
- Braschi, I., Ciavatta, C., Giovannini, C., Gessa, C., 2003. Combined effect of water and organic matter on phosphorus availability in calcareous soils. *Nutr. Cycl. Agroecosyst.* 67, 67–74.
- Brockway, D.G., 1983. Forest floor, soil, and vegetation responses to sludge fertilization in red and white pine plantations. *Soil Sci. Soc. Am. J.* 47, 776–784.
- Caravaca, F., Figueroa, D., Alguacil, M.M., Roldán, A., 2003. Application of composted urban residue enhanced the performance of afforested shrub species in a degraded semiarid land. *Bioresour. Technol.* 90, 65–70.
- Cerdà, A., 1996. Soil aggregate stability in three Mediterranean environments. *Soil Technol.* 9, 133–140.
- Cheng, H., Xu, W., Liu, J., Zhao, Q., He, Y., Chen, G., 2007. Application of composted sewage sludge (CSS) as a soil amendment for turfgrass growth. *Ecol. Eng.* 29, 96–104.
- Cogliastro, A., Domon, G., Daigle, S., 2001. Effect of wastewater sludge and woodchip combinations on soil properties and growth of planted hardwood trees and willow on a restored site. *Ecol. Eng.* 16, 471–485.
- Cox, D., Bezdicsek, D., Fauci, M., 2001. Effects of compost, coal ash, and straw amendments on restoring the quality of eroded Palouse soil. *Biol. Fertil. Soils* 33, 365–372.
- Dolgen, D., Alpaslan, M.N., Delen, N., 2004. Use of an agro-industry treatment plant sludge on iceberg lettuce growth. *Ecol. Eng.* 23, 117–125.
- Fox, G.A., 1993. Failure-time analysis: emergence, flowering, 1 survivorship, and other 2 waiting times. In: Schneider, S.M., Gurevich, J. (Eds.), *Design and Analysis of 3 Ecological Experiments*. Chapman & Hall, New York.
- Fuentes, D., Valdecantos, A., Cortina, J., Vallejo, V.R., 2007. Seedling performance in sewage sludge amended degraded Mediterranean woodlands. *Ecol. Eng.* 31, 281–291.
- Ganatsas, P., Spanos, I., 2005. Root system asymmetry of Mediterranean pines. *Plant Soil* 278, 75–83.
- Hall, J., 1999. Ecological and economical balance for sludge management options. In: Langenkamp, H., Marmo, L. (Eds.), *Proceedings of Workshop Acknowledgments on Problems Around Sludge*. European Commission (EUR 19657). Joint all copy Research Centre, Stresa (NO), Italy, pp. 155–172.
- Harrison, R.B., Gessel, S.P., Zabowski, D., Henry, C.L., Xue, D., Cole, D.W., Compton, J.E., 1996. Mechanisms of negative impacts of three treatments on nutrient availability. *Soil Sci. Soc. Am. J.* 60, 1622–1628.
- Henry, C.L., Cole, D.W., 1997. Use of biosolids in the forest: technology, economics and regulations. *Biomass Bioenerg.* 13, 269–277.
- Hernández, T., Moral, R., Pérez-Espinosa, A., Moreno-Caselles, J., Pérez-Murcia, M.D., García, C., 2002. Nitrogen mineralization potential in calcareous soils amended with sewage sludge. *Bioresour. Technol.* 83, 213–219.
- Jacobs, D.F., Timmer, V.R., 2005. Fertilizer-induced changes in rhizosphere electrical conductivity: relation to forest tree seedling root system growth and function. *New Forest* 30, 147–166.
- Jacobs, D.J., Rose, R., Haase, D.L., Alzugaray, O., 2004. Fertilization at planting impairs root system development and drought avoidance of Douglas-fir (*Pseudotsuga menziesii*) seedlings. *Ann. For. Sci.* 61, 643–651.
- Jones, J.B., Case, V.W., 1990. Sampling, handling, and analysing plant tissue samples. In: Westerman, R.L. (Ed.), *Soil Testing and Plant Analysis*, SSSA Book Series, vol. 3, Madison, WI, pp. 389–427.
- Keeney, D.R., 1982. Recommended biological index ammonium-nitrogen production under waterlogged conditions. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis*, Part 2, 2nd ed. American Society of Agronomy, Soil Sci. Soc. Am., Madison, WI, US, pp. 727–728.
- Keeney, D.R., Nelson, D.W., 1982. Nitrogen-inorganic forms. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis*, Part 2, 2nd ed. American Society of Agronomy, Soil Sci. Soc. Am., Madison, WI, US, pp. 643–698.
- Larchevêque, M., Ballini, C., Korboulewsky, N., Montes, N., 2006. The use of compost in afforestation of Mediterranean areas: effects on soil properties and young tree seedlings. *Sci. Total Environ.* 369, 220–230.
- Larchevêque, M., Montes, N., Baldy, V., Ballini, C., 2008. Can compost improve *Quercus pubescens* Willd establishment in a Mediterranean post-fire shrubland? *Bioresour. Technol.* 99, 3754–3764.
- Maestre, F.T., Bautista, S., Cortina, J., Díaz, G., Honrubia, M., Vallejo, V.R., 2002. Microsite and mycorrhizal inoculum effects on the establishment of *Quercus coccifera* in a semi-arid degraded steppe. *Ecol. Eng.* 19, 289–295.
- Marx, D.H., Berry, C.R., Kormanik, P.P., 1995. Application of Municipal sewage sludge to forest and degraded land. In: Karlen, D.L., Wright, R.J., Kemper, W.O. (Eds.), *Agricultural Utilisation of Urban and Industrial By-products*. ASA, CSSA, SSSA, Madison, WI, pp. 275–295.
- 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., Bermúdez, F., Machín, J., 1998. Influence of sewage sludge application on physical and chemical properties of Gypsissols. *Geoderma* 87, 123–135.
- Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon and organic matter. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis*, Part 3, Chemical Methods. SSSA-ASA, Madison, WI, pp. 1011–1069.
- Oliet, J.A., Planelles, R., Artero, F., Valverde, R., Jacobs, D.F., Segura, M.L., 2009. Field performance of *Pinus halepensis* planted in Mediterranean arid conditions: relative influence of seedling morphology and mineral nutrition. *New Forest* 37, 313–331.
- Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis*, Part 2, 2nd ed. American Society of Agronomy, Soil Sci. Soc. Am., Madison, WI, US, pp. 403–430.
- Pascual, J.A., García, C., Hernández, T., 1999. Comparison of fresh and composted organic waste and their efficacy for the improvement of arid soil quality. *Bioresour. Technol.* 68, 255–264.
- Pausas, J.G., Bladé, C., Valdecantos, A., Seva, J.P., Fuentes, D., Alloza, J.A., Vilagrosa, A., Bautista, S., Cortina, J., Vallejo, V.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.
- Pérez-Devesa, M., Cortina, J., Vilagrosa, A., Vallejo, V.R., 2008. Shrubland management to promote *Quercus suber* L. establishment. *For. Ecol. Manag.* 255, 374–382.
- Powers, R.F., 1980. Mineralizable soil nitrogen as an index of nitrogen availability to forest trees. *Soil Sci. Soc. Am.* 44, 1314–1320.
- Querejeta, I., Roldán, A., Albadalejo, J., Castillo, V., 1998. The role of mycorrhizae site preparation and organic amendment in the afforestation of a semiarid Mediterranean site with *Pinus halepensis*. *For. Sci.* 44, 203–211.
- 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, pp. 2087–2086.
- Quezel, P., 2000. Taxonomy and biogeography of Mediterranean pines (*Pinus halepensis* and *P. brutia*). In: Ne'eman, G., Trabaud, L. (Eds.), *Ecology, Biogeography and Management of Pinus halepensis and P. brutia Forest Ecosystems in the Mediterranean Basin*. Backhuys Publishers, Leiden, pp. 1–12.
- Ramsey, C.L., Jose, S., Brecke, B.J., Merritt, S., 2003. Growth response of longleaf pine (*Pinus palustris* Mill.) seedlings to fertilization and herbaceous weed control in an old field in southern USA. *For. Ecol. Manag.* 172, 281–289.
- Rhoades, J.D., 1982. Soluble salts. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis*, Part 2, 2nd ed. American Society of Agronomy, Soil Sci. Soc. Am., Madison, WI, US, pp. 403–430.
- Roldán, A., Querejeta, I., Albaladejo, J., Castillo, V., 1996. Survival and growth of *Pinus halepensis* Miller seedlings in a semi-arid environment after forest soil transfer, terracing and organic amendments. *Ann. For. Sci.* 53, 1099–1112.
- Rose, R., Ketchum, J.S., 2002. Interaction of vegetation control and fertilization on conifer species across the Pacific Northwest. *Can. J. For. Res.* 32, 136–152.
- Sardans, J., Peñuelas, J., Ogaya, R., 2008. Experimental drought reduced acid and alkaline phosphatase activity and increased organic extractable P in soil in a *Quercus ilex* Mediterranean forest. *Eur. J. Soil Biol.* 44, 509–520.
- Sort, X., Alcañiz, J.M., 1996. Contribution of sewage sludge to erosion control in the rehabilitation of limestone quarries. *Land Degrad. Dev.* 7, 69–76.
- Tarrasón, D., Ortiz, O., Alcañiz, J.M., 2007. A multi-criteria evaluation of organic amendments used to transform an unproductive shrubland into a Mediterranean *dehesa*. *J. Environ. Manag.* 87, 446–456.
- Topp, G.C., Davis, J.L., 1985. Measurement of soil water content using time-domain reflectometry (TDR): a field evaluation. *Soil Sci. Soc. Am. J.* 49, 19–24.
- Trubat, R., Cortina, J., Vilagrosa, A., 2008. Short-term nitrogen deprivation increases field performance in nursery seedlings of Mediterranean woody species. *J. Arid Environ.* 72, 879–890.
- USEPA (United States Environmental Protection Agency), 1995. *Process Design Manual. Land Application of Sewage Sludge and domestic septage*. EPA 625-R-95-001, Washington, DC.
- USEPA (United States Environmental Protection Agency), 1999. *Biosolids Generation, Use, and Disposal in the U.S.*, EPA 530-R-99-00, Washington, DC.
- Valdecantos, A., 2001. *Aplicación de fertilizantes orgánicos e inorgánicos en la repoblación de zonas forestales degradadas de la Comunidad Valenciana*. Ph.D. Thesis. Universidad de Alicante.
- 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.

- Vallejo, V.R., Bautista, S., Cortina, J., 2000. Restoration for soil protection after disturbances. In: Trabaud, L. (Ed.), *Life and Environment in the Mediterranean*. WIT Press, pp. 301–343.
- van den Driessche, R., Rude, W., Martens, L., 2003. Effect of fertilization and irrigation on growth of aspen (*Populus tremuloides* Michx.) seedlings over three seasons. *For. Ecol. Manag.* 186, 381–389.
- Yaalon, D.H., 1997. Soils in the Mediterranean region: what makes them different? *Catena* 28, 157–169.
- Zagas, T., Ganatsas, P., Tsitsoni, T., Hatzistathis, A., 2000. Influence of sewage sludge application on survival and early growth of forest species. In: Tsihrizis, V. (Ed.), *Proceedings of Thrace 2000. Protection and Restoration of the Environment*, vol. I, pp. 583–590.
- Zornoza, R., Mataix-Solera, J., Guerrero, C., Arcenegui, V., Mayoral, A.M., Morales, J., Mataix-Beneyto, J., 2007. Soil properties under natural forest in the Alicante Province of Spain. *Geoderma* 142, 334–341.