Full title: CARBON BALANCE AS AFFECTED BY BIOSOLID APPLICATION IN REFORESTATIONS

Running head: C BALANCE AS AFFECTED BY BIOSOLID APPLICATION IN REFORESTATIONS

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Keywords: Carbon sequestration, litter, *Pinus halepensis* Mill., root biomass, soil organic carbon

Abstract

Soils of Mediterranean drylands are characterized by a low fertility and organic matter content because of past land use and disturbances regime. The restoration of these degraded lands faces at the same time problems related to water scarcity and the unpredictability of precipitations with problems with soil physical, chemical and microbiological properties. Organic amendments may help to improve soil properties and, consequently, enhance planted seedling establishment and performance. In this study, we assessed the C balance of three Mediterranean areas planted with *Pinus halepensis* Mill. seedlings with different treatments of biosolid application. The assessment was conducted at different times after the establishment of treatments and the C dynamics are discussed. We considered three biosolid types (air-dried, fresh sludge, and composted sludge) in application doses ranging from 10 to 320 Mg (d.w.) ha^{-1}. We quantified basal area, pine biomass, biomass of spontaneous vegetation, litter, root density and soil organic matter. All three experimental restoration studies improved restoration success in terms of basal area (ranging from 15 to 300 %), especially in composted biosolid at 30 Mg (d.w.) ha^{-1}, while litter and, especially, root biomass increased with all biosolid treatments and time since application. Soil organic C was

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higher in application doses above 30 Mg (d.w.) ha^{-1} due to the organic matter applied with the sludges. The increment in C sequestration rates associated to these restoration treatments ranged between 0.046 to 0.293 kg C m^2 yr^{-1}. These results confirm the suitability of organic amendments as restoration technique in Mediterranean degraded drylands.

Keywords: Carbon sequestration, litter, *Pinus halepensis* Mill., root biomass, soil organic carbon

1. Introduction

The role of forest and shrubland ecosystems in the C sequestration from the atmosphere is unquestionable (Jobbagy & Jackson, 2000). Wildfires are one of the most important and recurrent threats of Mediterranean landscapes affecting roughly half a million of hectares every year (Khabarov *et al.*, 2016). This disturbance has direct consequences on global warming processes by different ways (Thornton, 2002; Law *et al.*, 2004; Narayan *et al.*, 2007). Their effects may last for variable periods of time, depending on fire severity and frequency, type of burned vegetation and its recovery rate (Dore *et al.*, 2008; Mayor *et al.*, 2016) and might never recover soil C storage if the regeneration of aboveground plant community fails (Kaye *et al.*, 2010). Over-grazing represents another important disturbance in Mediterranean landscapes (Perevolotsky & Seligman, 1998) that affects carbon balance in ecosystems by reducing leaf area index, biomass and other plant properties related to physiological performance (Schmitt *et al.*, 2010).

The promotion of afforestations of set-aside agricultural lands and reforestations of degraded natural landscapes is urged by national and international directives on climate change mitigation (IPCC, 2014). These restoration programs should incorporate techniques and actions aimed both at establishing a more developed plant cover with disappeared or hampered species, reducing the risk of further degradation turning the source areas into sinks that retain resources, and increasing the recovery capacity of the ecosystem in case of disturbances (Valdecantos *et al.*, 2009).

Reforestation is one of the most common restoration techniques used to recover plant cover in a short-medium term (Vallejo *et al.*, 2012). Soil preparation before planting is needed to decrease seedling transplant shock and the impacts of initial drought events in harsh environments (Chirino *et al.*, 2009). This soil alteration exposes organic materials to microbial populations resulting in the mineralization and decomposition of this pool of sequestered carbon (Löf *et al.*, 2012). As a consequence, afforestations and plantations lead to a temporal depletion of the soil C pool (Jandl *et al.*, 2007) and this effect may last even decades until the inputs by litterfall and root turnover overcome the outputs by heterotrophic respiration and leaching. However, decomposition rates of soil organic matter in Mediterranean ecosystems is relatively low because water scarcity (Bottner *et al.*, 2000) and, in turn, Mediterranean forests represent efficient sinks with high C sequestration potential (Valentini *et al.*, 2000).

Organic amendments constitute an interesting opportunity for enhancing organic matter and fertility of agricultural and forest soils in dry and semiarid environments (Querejeta *et al.*, 2000; Caravaca *et al.*, 2003) that can last up to 10 years after the application (Pascual *et al.*, 2000). In general, they enhance growth of planted seedlings (McNab & Berry, 1985; Roldan *et al.*, 1996; Garcia-Franco *et al.*, 2014) but it rarely enhances the survival of planted seedlings or it even reduces their successful establishment (Jiménez *et al.*, 2007; Larchevêque *et al.*, 2008; Piñeiro *et al.*, 2013; Hueso-González *et al.*, 2016). In dryland ecosystems, biosolids might intensify specific stress factors such as intense droughts that, together with increases in soil electrical conductivity and root competition, negatively affect water availability to the planted seedlings (Fuentes *et al.*, 2010).

Biosolid amendments often increase soil microbial activity (Ros *et al.*, 2003) and nutrient availability and also improve soil physical properties (García-Orenes *et al.*, 2005). Attending to the carbon cycle, sludge applications imply a direct input of exogenous carbon to the soils. Then, the behaviour of the soil organic matter depends on his physicochemical composition and the distribution between labile and recalcitrant pools of C determining the portion used by microorganisms as energy and the portion that contribute to the pool of humic-like soil stable substances (González-Ubierna *et al.*, 2012; Ojeda *et al*., 2015). The increase of activity of soil microbial populations promotes the rate of organic matter decomposition and mineralization and, hence, the amount of CO_2 emitted to the atmosphere (Fontaine *et al.*, 2004) but at the same time it increases plant productivity and C fixation both, at medium and a long term (Pascual *et al.*, 2000; Hernández *et al.*, 2015). The question is whether the use of biosolids in reforestations increases C fixation in higher extent than C emission, resulting in a positive balance in C sequestration.

Pinus halepensis Mill. is a fast-growing drought tolerant species covering 6.800.000 ha in the Mediterranean region (Farjon, 2010). It is the prevailing tree in dry and semiarid areas and broadly used in reforestation and restoration activities (Pausas *et al.*, 2004) because the high rates of seedling survival and growth (Barberá *et al.* 2005; Fuentes *et al.*, 2007) and its plasticity to changes in the soil environment (Broncano *et al.*, 1998). Nevertheless, its use in high water-stressed semi-arid systems may be detrimental to stimulate successional processes (Bellot *et al.*, 2004) and their adaptation may not be sufficient for future climatic scenarios (García de la Serrana *et al.*, 2015).

In this paper we conducted and approach to evaluate the short-medium term effect on the carbon balance of the applications of different doses and types of biosolids in reforestation sites of *Pinus halepensis* Mill. in areas affected by wildfires and grazing. This approach was carried out in plots established with the objective of testing the suitability of applying biosolids in the reforestation of Mediterranean degraded areas.

2. Materials and Methods

Three different reforestation sites that included the application of biosolids were established in highly disturbed sites (fires and grazing) in the Valencia region (E Spain) (Table 1). All the plots were located in slopes lower than 20%, on shallow, basic soils, developed from limestone and marl (*Cambisols*) and shared similar vegetation composition and structure: a sparse shrubland dominated by obligate seeder species such as *Rosmarinus officinalis* L., *Ulex parviflorus* (Pourr.), and *Cistus* spp., and lower presence of resprouters like *Q. coccifera*. Spontaneous recruitment of trees (*Pinus halepensis* Mill., *P. pinaster* Ait. and *Q. ilex* L.) was scarce. The mean annual temperature and precipitation of the three sites ranged from 12.0 to 15.1ºC and 357 to 479 mm, respectively (Table 1).

In all the sites we planted 1-year-old seedlings of *Pinus halepensis* Mill. as the target species that disappeared locally due to the short fire return intervals. A fully description of the sites may be found in Fuentes *et al.* (2007, 2010) and Valdecantos *et al.* (2011), including the description of initial soil and organic amendments characteristics and issues related to heavy metals and their potential effects on the introduced seedlings.

2.1.Field sites

Experiment 1. Ayora site.

Planting holes were dug by means of a 20-cm diameter by 40 cm depth auger in three 0.3 ha experimental plots 2 km away from each other. Experimental treatments consisted in the application of liquid (L10) and air-dried biosolids (D10) from a domestic waste water treatment plant at rates of 10 Mg dry weight ha^{-1} (ca. 0.160 kg per planting hole), and unamended control holes (C) (Table 1). Dry biosolid was air-dried until moisture content was ca. 15% and applied on the topsoil of the holes after planting whereas liquid biosolid (1.4% dry matter) was applied before the plantation at the deepest part of the hole. We planted 50 (C and L10 treatments) or 25 (D10) one-year old seedlings of *Pinus halepensis* Mill. on separate holes and treatments were interspersed within plots. The assessment of C pools was carried out 10 years after planting.

Experiment 2. Zarra site.

A backhoe excavator was used to dig planting holes in a 1.46 ha demonstration project. Anaerobically digested sewage sludge (20% of dry matter) was mixed with the soil at 0, 7.5 and 14.5 kg dry weight (d.w.) per planting hole (equivalent to 0, 220 and 310 Mg dry weight ha^{-1}) (Table 1). The planting holes of each treatment were patchy distributed along the whole experimental area due to constraints of the machinery employed in loading, transporting and applying the biosolid. Seventy-five 1-year-old seedlings of *Pinus halepensis* Mill. were planted per treatment and plot. The assessment of C pools was carried out 7 years after planting.

Experiment 3. Enguera site.

A backhoe excavator dug planting holes with a minimum of 30 cm depth in a single location. We used different biosolids from a domestic wastewater treatment plant: Composted biosolids (C) or air-dried biosolids (D) were mixed *in situ* with the soil of the planting holes at doses ranging between 0 to 0.945 kg d.w and 0 to 0.960 kg d.w. per planting hole of dry biosolids and compost, respectively (equivalents to 0, 15, 30 and 60 Mg dry weight ha^{-1}). Treatments were interspersed within the experimental plot (Table 1). Soon after site preparation we planted thirty 1-year-old *Pinus halepensis* Mill. seedling per biosolid type and dose. The assessment of C pools was carried out 4 years after planting.

2.2.Monitoring C pools

We carried out an extensive sampling in all reforestation plots for assessing the amount of C stored in the different compartments of the sites. Time since planting was 117, 81 and 45 months for the Ayora, Zarra and Enguera experimental designs, respectively (10, 7 and 4 years hereafter). We focussed on i) the planted individuals of *Pinus halepensis* Mill. (target seedlings), ii) the natural spontaneous vegetation colonizing the planting hole, iii) the organic horizon on the soil surface, iv) the belowground biomass in the first 20 cm of the soil, and v) the organic carbon in the mineral soil (0-20 cm). We evaluated seedling survival rate and morphology (basal diameter, BD) in every treatment and design. We estimated pine aboveground biomass (AB) by applying the following allometric equation released from own data: $AB = 65.294*BD^2 + 19.193*BD$ (r^2=0.9645, n=56, range of ages: 1-9 years). In 10 planting holes per treatment (5 with living plant and 5 where the planted seedling died) we harvested all the aerial biomass of natural vegetation in 0.5 x 0.5 m quadrats. Litter and soil probes (0-20 cm) were collected in the same planting holes in 0.25 x 0.25 m quadrats to determine root density and soil organic carbon. Two soil probes were collected in each planting hole and mixed in a composite sample per hole. Litter samples were carried to the lab and gently separated plant materials from soil particles and oven dried for 48 h at 70ºC and weighted. Identical procedure was applied to roots extracted from soil samples, estimating root density as the fraction between root biomass and the volume of the probe. Biomass carbon content from different fractions (i.e. aerial biomass of natural vegetation, litter and roots) was calculated by multiplying by a standard coefficient of 0.5 (IPCC, 1996; Schlesinger, 1997). Soil organic matter (SOM) was determined by potassium dichromate oxidation in the Walkley-Black method (Nelson & Sommers, 1996) and organic C was calculated by multiplying SOM by the van Bemmelen constant of 0.58. The approach to the bulk soil C content was conducted by assuming a soil bulk density value of 1.17 g cm^{-3} commonly found in these carbonated Mediterranean soils determined by own data, and a mean surface of the planting holes of 0.16 m^2 in the Ayora and Enguera designs, and 0.39, 0.35 and 0.46 m^2 for Z0, Z220 and Z310, respectively, in the Zarra design. In each experimental design we also calculated the basal area of all treatments by summing the basal sections of all surviving pines (as an integrated value of survival and growth). The best treatment per experimental design was selected according to the highest basal area data. All data were extrapolated to an initial planting density of 800 seedlings ha^{-1} to standardize. For the final C balance, we summed all the C fractions in each planting hole and calculated separately C pools for holes with pines alive and dead. These figures were weighted by the survival and mortality rate of each treatment, respectively, for an overall estimation of each experimental treatment. Final data are expressed in kg C m^{-2}. To determine the annual rate of C content (kg C m^{-2} yr^{-1}) we subtracted to each treatment the soil C content of their respective control treatments and divided by the years since planting.

2.3.Statistical analysis

In every individual experimental design, we carried out a log-linear analysis for seedling survival data and analysis of variance with one fixed factor (sludge treatment) at different levels for pine growth, and with a second fixed factor (planted seedling survival, dead or alive) for all other variables. We also included a random factor (plot, at three levels) in the Ayora design. Data were transformed when needed to avoid heterocedasticity. However, it is explicitly stated in the text those cases in which variance homogeneity was not achieved after data transformation. These analyses were performed with SPSS v 15.0 statistical package (SPSS Inc., Chicago, USA).

3. Results

Experiment 1 – Ayora

Ten years after the application of low doses of liquid or air-dried biosolid in the planting hole minor changes were still significant both on the plant and soil compartments (Table 2). Proliferation of roots of both the planted seedling and the natural vegetation in the planting hole significantly increased with the application of air-dried biosolids. However, all other soil and plant C fractions showed a trend to increase with sludge application in relation to controls, especially in the AL10 treatment: biomass of the planted pines and that of natural vegetation increased in 29.0 and 45.1%, respectively, in AL10 amended holes in relation to unfertilized ones. Less pronounced increases were observed in litter accumulation and soil organic C (Table 2). Survival of *Pinus halepensis* Mill. seedlings was not affected by treatments ten years after planting, ranging between 70.1 and 80.2%. The presence of living individuals of *P. halepensis* in the planting hole significantly increased litter accumulation on soil surface as compared to holes where seedlings died (1042 vs 501 g m^{-2}, respectively). On the other hand, soil organic C was marginally significant lower in holes with living plants than in those with dead pines (3.11 vs 3.31 %, respectively). In this experiment, there was an important and significant effect of the planting plot on all tested variables except litter accumulation, more relevant than the sludge application treatments.

The total amount of C accumulated in all studied fractions ten years after planting, considering seedling survival rates of the corresponding treatments, ranged between 8.38 and 8.84 Kg C m^{-2}. The three treatments showed a similar distribution of C between fractions, the highest amount (≈ 85%) stored in the mineral soil (0-20 cm) (Fig. 1). Excluding the soil C fraction, pine biomass accounted for 25.9 to 32.5% of the rest of C pools while litter, spontaneous vegetation and roots in the planting hole accumulated 36.4, 7.9 and 25.8%, respectively (Fig. 2).

Experiment 2 – Zarra

Contrary to Ayora site, high application rates of biosolids showed significant effects in litter accumulation and soil organic C (Table 3). Litter horizon was higher in the sludge treatments than in controls, with relative increments of 94 and 75% in the Z310 and Z220 treatments, respectively. Soil organic matter concentration increased 29.3% (from 3.75 to 4.85% in average) in amended soils in relation to controls. The highest application dose increased a 35% the pine biomass compared to control one. Nevertheless, it is important to highlight that this treatment (Z310) reduced significantly the survival rates of the planted seedlings in relation to the control treatment. As well as in the Ayora site experiment, the accumulation of litter in the holes was significant and positively affected by the presence of alive individuals of pine (399 vs 241 g m^{-2}).

Total C pool in the Zarra experimental design seven years after the establishment of the plantation summed 5.87, 6.95 and 7.21 Kg C m^{-2} in the Z0, Z220 and Z310 treatments, respectively. Excluding soil C content (ranging between 89.6 and 90.4% of the total; Fig. 1), pines were the second source of C to the total pool (39.3-42.0%) followed by roots (24.5-29.7%), litter (19.8-29.8%) and, in a lesser extent, spontaneous vegetation (4.2-8.8%) (Fig. 2). Although pines were bigger in the Z310 treatment, the significantly lower survival rate of seedlings in this treatment produced less total C in this fraction than in the Z220 treatment.

Experiment 3 – Enguera

The comparison between different doses and types of biosolids revealed that four years after planting air-dried biosolid released more pronounced effects than composted biosolid. Both dry and composted biosolids applied at 15 and 30 Mg (d.w.) ha^{-1} significantly promoted the growth of the planted seedlings as compared to controls (Table 4). Other fractions such as litter and roots showed a positive trend with the application of dry sludge and, in a lesser extent, with composted one. Only the highest dose of dry sludge (D60) marginally increased the soil organic matter concentration but reduced pine seedling survival in relation to the intermediate application doses. In the case of composted sludge, seedling survival was not significantly affected by any doses but, contrary to dry sludge significantly, increased root density in the 0-20 cm soil horizon in holes with alive pine seedlings in relation to those with dead pines.

Carbon accumulation after 4 years since planting ranged between 6.29 and 8.67 Kg C m^{-2}. Different type of biosolids had similar effect on total C accumulation and it was proportional to the doses applied. Soil organic matter ranged from 92.4 to 95.9% of total C pool considered in the balance. Again, when excluding soil carbon, the main contribution to the C pool came from the roots (84.6% in Dry sludge and 80.6% in Compost) followed by the litter fraction (8.5 and 11.8 %, respectively), the spontaneous vegetation (3.7 and 5.2%) and the introduced pines that represent the smallest fraction (3.1 and 2.4%, respectively). This low contribution of the pine biomass was due to the general high mortality and low growth rates of seedlings in this experimental site, intensified by the occurrence of two events of extreme drought.

3.1.Annual rates of C sequestration in most successful treatments

Treatments that showed the best combination of plant survival and growth, measured as basal area, were AL10 treatment in Ayora site (62 m^2 of basal area vs 53 m^2 in A0), Z220 in Zarra site (46 vs 40 m^2 in Z0) and EC30 and EL30 (1.93 and 2.83 m^2, respectively, vs 0.69 m^2 in E0) in Enguera site. Application of these treatments increased the total C accumulation up to 0.156 kg C m^{-2} yr^{-1} in Ayora, 0.240 kg C m^{-2} yr^{-1} in Zarra and 0.256-0.359 kg C m^{-2} yr^{-1} in Enguera (compost and dry-sludge, respectively) in relation to their respective controls, including the difference in SOC between treatment and the respective control (Fig. 3). These values represent an increment of 42% in Ayora and approximately 3.0, 4.5 and 5.5-fold increase in the other experimental sites, respectively.

However, these treatments were not always those that maximized C sequestration as compared to their respective controls. Considering the total C accumulation in time as a priority target to restoration, the best results in each site in relation to their respective control ranged from 0.156 and 0.619 kg C m^{-2} yr^{-1}, having into account all the C fractions including C in mineral soil.

4. Discussion

Afforestation of degraded and unproductive agricultural areas has been proposed as effective actions to mitigate the impacts of climate change (IPCC, 2014). However, afforestations may produce beneficial, neutral or even negative effects on the final C balance (Guo & Gifford, 2002; Vesterdal et al., 2002; Nosetto *et al.*, 2006) being this final result especially conditioned by soil characteristics (Muñoz-Rojas *et al.*, 2015; Segura et al., 2016), the planted species (Gao *et al.*, 2014) and the previous land use of the afforested area (Lal, 2005; Laganière *et al.*, 2010). In general, reforestation actions promote an initial reduction of the amount of C stored in the soil, more pronounced in shallow horizons, as C inputs from litter are still low and decomposition is enhanced because site preparation implies alteration of soil horizons and new C-based substrates are susceptible to be mineralized and respired by microbial populations (Paul *et al.*, 2002; Jandl *et al.*, 2007). This especially happens when heavy site preparation techniques are used during reforestation (Garcia-Franco *et al.*, 2014).

In the three experimental designs of our study, individual biomass of planted *Pinus halepensis* Mill. increased with all sludge treatments (Tables 2, 3 and 4), but not always significantly.

The effect of organic amendments on the soil-plant system will depend of factors such as the type and dose of amendment, the stabilization degree of the organic matter applied (Hernández *et al.*, 2002), the soil and climatic properties of the reforested site or the planting technique used (Piñeiro *et al.*, 2013; Garcia-Franco *et al.*, 2014). The availability of sources of carbon to be stabilized into the soil matrix or to be accessible to decomposers will depend on the soil texture and pH (Plante *et al.*, 2006). The degree of incorporation into the soil will also determine the role of the different stabilization mechanisms and the turnover times of the organic matter (Lützow *et al.*, 2006). In Mediterranean drylands, the beneficial effect of organic matter applications on plant growth, especially by reducing deficiencies of limiting nutrients (Valdecantos *et al.*, 2011), is higher than that provided by other restoration techniques (Barberá et al., 2005; Valdecantos et al., 2014). However, nutritional benefits are surrogated to water availability which, ultimately, determines plant survival in these environments (Valdecantos et al., 2014; Fuentes et al., 2017) and, hence, the C storage potential in plant biomass.

Despite the strong effect on the net C soil storage (Lal, 2005, Hernández et al., 2015), the use of high doses of biosolids optimized to recover the degraded microbiological properties of the soil may have negative effects in the vegetation when is combined with intense droughts. The increase of soil electrical conductivity (Fuentes *et al.*, 2010), physical problems in the soil when fresh sludges dry out (Fuentes *et al.*, 2007) or the excessive development of potentially competing spontaneous vegetation (Fuentes et al., 2010; Berry, 1979) are among the ultimate causes that determine seedling mortality when biosolids are applied in the restoration of dry and semiarid lands using punctual soil preparation techniques as a way to avoid excessive soil removal and damage to natural vegetation.

At adequate doses, positive impacts of this technique such as increases of aboveground biomass (Rincón *et al.*, 2006; Fuentes et al., 2007), or improving organic matter and nutrient content, structure and microbiological activity of soils (Querejeta et al., 2000; Tarrasón *et al.*, 2007) may counterbalance the neutral to negative impact on seedling survival. The biochemical effects of relatively small doses can last up to three years enhancing the development of both the introduced seedlings and the spontaneous vegetation, taking advantage of the most recalcitrant part of the added organic matter (Pascual *et al.*, 1999). Our study focused on the surface affected by the planting holes that, together with the low standard planting density (800 seedlings ha^{-1}) and the low productivity of the planting sites (Campo *et al.*, 2007), resulted in a relatively low C storage potential in most of the studied fractions (Figure 2). Taking into account the different survival rates of the planted pines in all the experimental treatments, C sequestration in the pine fraction is enhanced by biosolids as compared to their respective unamended controls. Garcia-Franco et al. (2014), 20 years after the establishment of the plantation, observed a 4-fold increase in the fraction of C stored in the aboveground biomass of afforested *Pinus halepensis* Mill. individuals when the plantation received 10 kg m^{-2} of composted urban refuse and were combined with heavy mechanical soil preparation. In our study, differences to the control in the C stored in pine biomass were higher in the experiments that were established earlier while changes in C in roots were more evident in intermediate application rates. The highest application dose produced negative rates of C storage in comparison to its control in both spontaneous vegetation and roots mostly associated to excessive soil salinity (Fuentes *et al.*, 2007). Our assessment covers different situations during the first ten years after planting, the period when soil C usually decreases, especially due to site preparation, while increased C sequestration is expected to occur from this moment onwards (Paul *et al.*, 2003).

Litter, natural vegetation and root density did not show a consistent pattern associated to the applications of sludge. Garcia-Franco et al. (2014) reported larger inputs of plant litter into the surface soil underneath amended than in unamended plants resulting in a gradual improvement of soil physical properties associated to the application of organic amendments. Organic amendments tended to increase litter accumulation in the three studied experiments, but the presence of alive pine individuals in the planting hole had a stronger influence in the litter accumulation being proportional to the stand age.

The sink effect of the planting hole increasing the soil surface roughness favouring the organic matter accumulation (Fuentes *et al.*, 2017), together with the presence of the surviving pines, resulted in significant differences of litter accumulation between holes where the planted pines survived or died. On the contrary, the most recent experiment in Enguera site released a trend to higher litter accumulation and natural vegetation in the planting holes where planted pines died than in holes with living pines. This could be due to the severe drought suffered in E Spain the years before sampling (Llasat *et al.*, 2009) that resulted in a sharp increase of vegetation senescence and death, especially in the highest application doses where root competition was more intense (Fuentes *et al.*, 2010). In both cases, the litter accumulation in functional planting holes, from the structural point of view, allows a modification on the sink-source pattern at the slope scale that increase the chance to C storage. This effect could be optimized by forcing the concentration of resources (water, seeds, fine and coarse organic matter, etc.) in the planting hole by redirecting the surface water fluxes (Fuentes *et al.*, 2017). Carbon accumulated in root fractions showed a trend to be higher in sludge amended planting holes than in control ones. The benefit of amendments was more pronounced in root-C than in litter-C fractions and this could have a longer term impact on soil C sequestration as it is mostly driven by roots rather than by litter (Hu *et al.*, 2016). Nevertheless, in most forests, the organic fraction present in surface detritus and roots are the lesser amount of the whole C pool as compared with aboveground biomass and mineral soil organic matter (Fahey *et al.*, 2010).

Annual rates of C sequestration in our study ranged between 0.066 and 0.359 kg C m^{-2} yr^{-1}. These overall values may seem low as compared to other reforestation studies in drylands (Nosetto *et al.*, 2006) but are within the range of *Pinus halepensis* Mill. reforestations in dry and semiarid Mediterranean areas (Garcia-Franco *et al.*, 2014; Grünzweig *et al.*, 2003). In addition, as the target of our approach was to assess whether the biosolid application treatments improved C storage in relation to traditional planting, the rates calculated in our study considered the soil C fraction just as the difference between the treatment and its respective control representing an underestimation of the overall C sequestration rates.

5. Conclusions

Despite the low C storage potential of the sites, the annual rates of C sequestration found in our study (in the range 0.066-0.359 kg C m^{-2} yr^{-1}) are consistent with those observed in similar reforested areas in Mediterranean drylands. Biosolid application enhanced the C sequestration in the aboveground fractions of plants at the short-medium term. Soil preparation enhanced the sink effect and thus, the final C sequestration, but abiotic factors like intense drought events have a great influence in the final balance of C storage. The pool of C accumulation in litter and, especially, roots fractions represented the main C storage compartment and tended to increase in biosolid-amended planting holes. This might represent a longer-term impact of biosolids in C sequestration. The application of biosolids, when applied at adequate doses, represents an improvement in the C balance in the reforestation of degraded Mediterranean sites.

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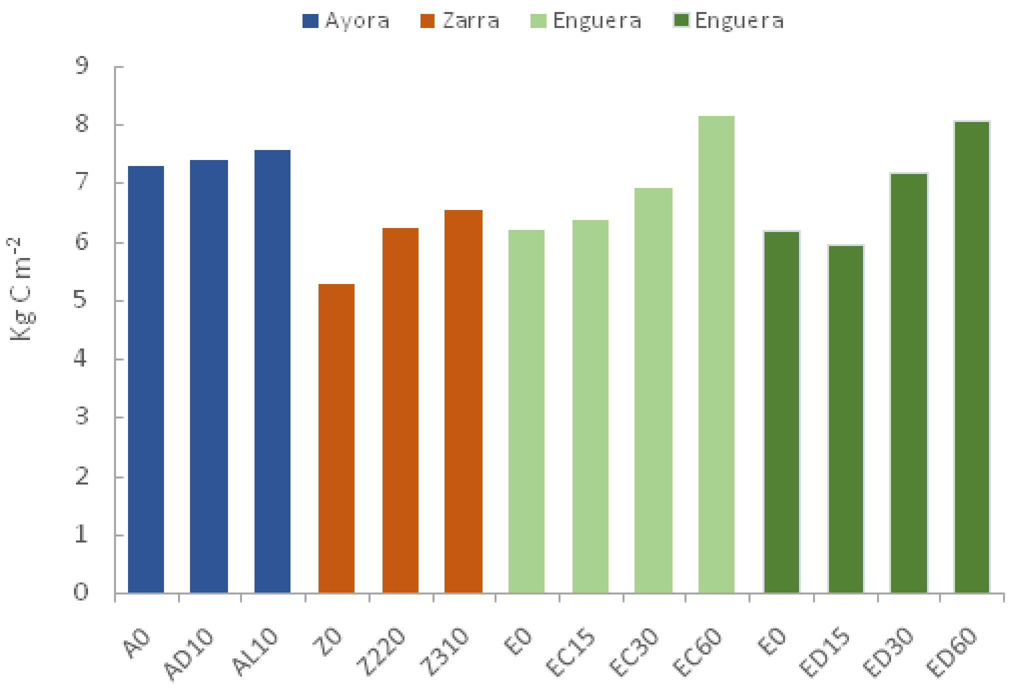

Figure 1. Carbon accumulation in the uppermost soil horizon (0-20 cm) ten (Ayora), seven (Zarra) and four (Enguera) years after planting in control and amended treatments in three different experimental sites. See Table 1 for treatment abbreviation.

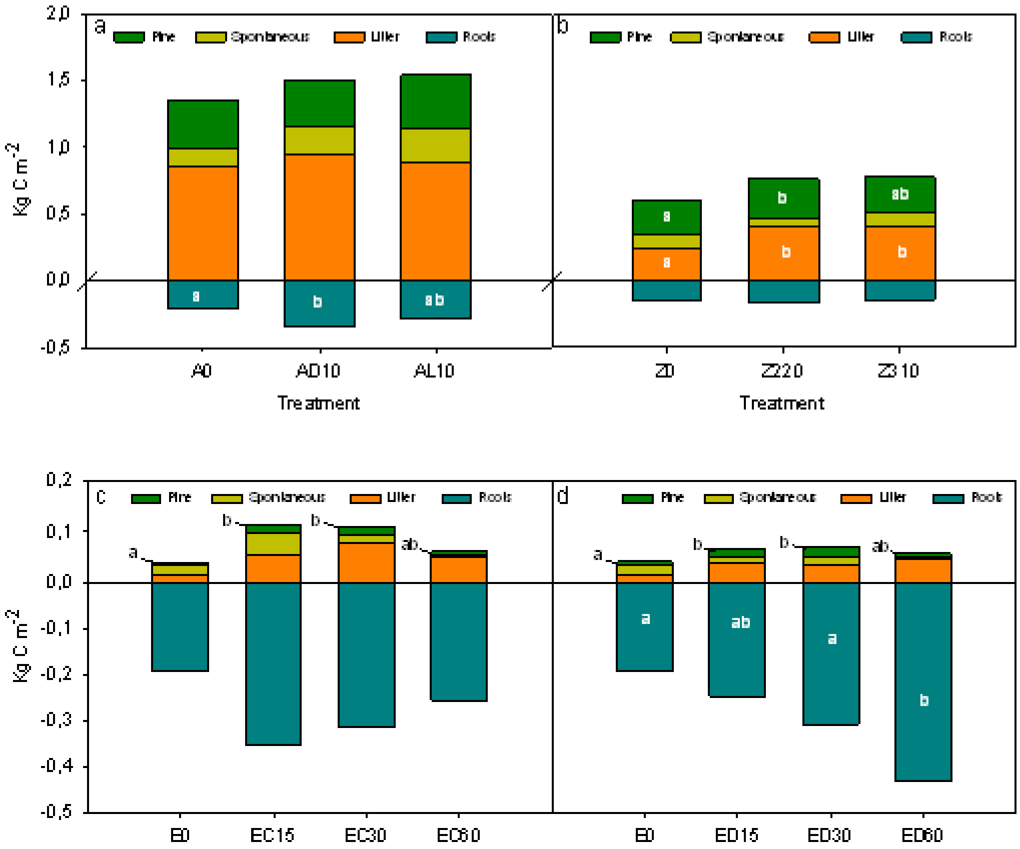

Figure 2. Carbon accumulation in the different biomass fractions (planted pine, spontaneous vegetation, litter and roots) after ten (a, Ayora), seven (b, Zarra) and four (c and d, Enguera) years since planting in control and amended treatments in three different experimental sites. Negative values indicate belowground fractions. See Table 1 for treatment abbreviation.

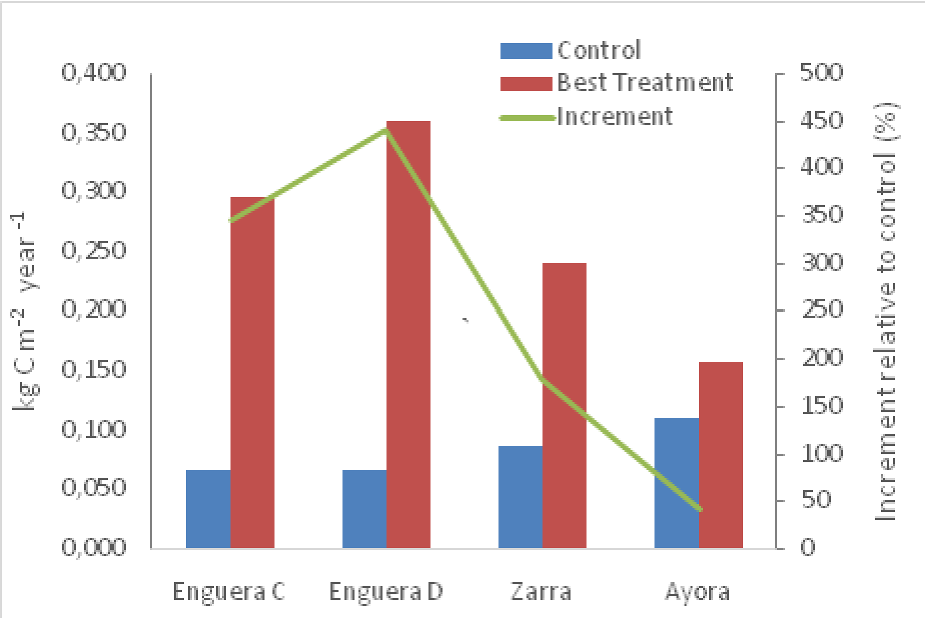

Figure 3. Annual rates of C accumulation in the control and the most success treatments on each experimental site: 30 Mg ha-1 of compost and dry sewage sludge in Enguera, 220 Mg ha-1 of fresh sewage sludge in Zarra, and 10 Mg ha-1 of liquid sludge in Ayora. The % of increment relative to control is shown on the right axis (green line).

Table 1. Summary of the three experimental designs.

	Ayora	Zarra	Enguera
Number of plots	3	1	1
Type of anaerobically digested biosolid	Dry and Liquid	Fresh	Dry and Composted
Application rate (Mg d.w. ha^{-1})	(D or L) 0, 10	0, 220, 310	(D or C) 0, 15, 30, 60
Treatment abbreviation	A0, AD10, AL10	Z0, Z220, Z310	E0, ED15, ED30, ED60, EC15, EC30, EC60
Number seedlings per treatment	75-150	75	30
Disturbance (years since last fire at planting)	Wildfires (6 yrs)	Grazing	Wildfires (3 yrs)
Mean annual temperature (ºC)	15.1	12.0	12.7
Mean annual precipitation (mm)	357	406	479

Table 2. Mean (and standard error) of seedling survival and morphology, and biomass of the different fractions ten years after the establishment of the Ayora experiment. The variables not related to the planted seedlings are separated for holes where the introduced plant was dead or alive at the moment of sampling. Results of log-linear (seeding survival) and ANOVAs are shown for the experimental factors Treatment (control, dry and liquid sludge at 10 Mg ha^{-1}), Plant Survival (alive and dead) and planting Plot (3 spatially replicated blocks). In bold, significant differences ($p<0.100$). Different letters correspond to significant differences in the Treatment Factor.

	Survival (%)	Basal Diameter (mm)	Pine Biomass (g)	Litter ($g\ m^{-2}$)		Natural Vegetation ($g\ m^{-2}$)		Roots ($mg\ cm^{-3}$)		Soil Organic Matter (%)	
				Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
A0	79.6 ± 1.1	32.5 ± 1.5	897.5 ± 82.1	936 ± 95	560 ± 111	112.2 ± 29.8	221.2 ± 46.7	2.23 ± 0.29	1.55 ± 0.32a	3.14 ± 0.22	3.01 ± 0.33
AD10	73.8 ± 1.6	33.7 ± 2.3	929.2 ± 112.7	1128 ± 121	454 ± 105	242.2 ± 73.0	118.8 ± 27	3.66 ± 0.74	2.61 ± 0.47b	2.98 ± 0.24	3.65 ± 0.21
AL10	69.9 ± 0.8	37.7 ± 1.7	1155.6 ± 102.3	1062 ± 126	488 ± 95	257.1 ± 87.0	229.0 ± 54.3	3.06 ± 0.49	2.18 ± 0.47ab	3.21 ± 0.24	3.27 ± 0.29
Treatment	χ^2=4.554 p=0.103	F=3.501 p=0.122	F=3.501 p=0.122	F=0.182 p=0.842		F=0.805 p=0.508		**F=10.070 p=0.025**		F=0.348* p=0.725	
Plant Survival	--	--	--	**F=36.463 p=0.026**		F=0.813 p=0.461		F=1.487 p=0.347		**F=8.587 p=0.090**	
Plot	**χ^2=53.032 p<0.001**	**F=15.854 p=0.005**	**F=15.854 p=0.005**	F=2.574 p=0.286		F=4.025 p=0.190		F=1.637 p=0.367		**F=22.664 p=0.075**	

*: variance homogeneity was not achieved.

Different letters in the same column indicate significant differences between treatments, pooling planting holes with alive and dead seedlings.

Table 3. Mean (and standard error) of seedling survival and morphology, and biomass of the different fractions six years after the establishment of the Zarra experiment. The variables not related to the planted seedlings are separated for holes where the introduced plant was dead or alive at the moment of sampling. Results of log-linear (seeding survival) and ANOVAs are shown for the experimental factors Treatment (Z0, Z220 and Z310 sludge treatments at doses equivalent to 0, 220 and 310 Mg ha^{-1}, respectively) and Plant Survival (alive and dead). In bold, significant differences ($p<0.100$). Different letters correspond to significant differences in the Treatment Factor.

	Survival (%)	Basal Diameter (mm)	Pine Biomass (g)	Litter ($g\ m^{-2}$)		Natural Vegetation ($g\ m^{-2}$)		Roots ($mg\ cm^{-3}$)		Soil Organic Matter (%)	
				Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Z0	76.4 ± 2.2a	28.9 ± 0.9a	657.5 ± 37.8a	270.3 ± 40.9	138.2 ± 24.0a	123.4 ± 31.0	49.0 ± 12.1	1.65 ± 0.35	1.13 ± 0.31	3.99 ± 0.40	3.52 ± 0.45a
Z220	75.7 ± 2.1ab	31.2 ± 1.4b	770.1 ± 71.8ab	455.6 ± 73.7	260.1 ± 38.1b	61.2 ± 18.2	60.4 ± 18.7	1.75 ± 0.36	1.42 ± 0.30	4.35 ± 0.46	5.32 ± 0.55b
Z310	60.7 ± 7.5b	34.0 ± 1.6ab	888.0 ± 72.5b	470.5 ± 64.7	323.5 ± 61.8b	90.5 ± 19.2	95.5 ± 26.6	1.66 ± 0.28	1.12 ± 0.13	4.58 ± 0.54	5.15 ± 0.69b
Treatment	**χ^2=6.392 p=0.041**	**F=4.204 p=0.016**	**F=4.149 p=0.017**	**F=11.988 p<0.001**		F=1.234 p=0.296		F=0.449 p=0.641		**F=2.975 p=0.057**	
Plant Survival	**--**	**--**	**--**	**F=16.186 p<0.001**		F=1.755 p=0.189		**F=3.039 p=0.087**		F=0.713 p=0.401	

Different letters in the same column indicate significant differences between treatments, irrespective of whether the planted pine was dead or alive.

Table 4. Mean (and standard error) of seedling survival and morphology, and biomass of the different fractions four years after the establishment of the Enguera experiment. The variables not related to the planted seedlings are separated for holes where the introduced plant was dead or alive at the moment of sampling. Results of log-linear (seeding survival) and ANOVAs are shown for the experimental factors Treatment (0, 15, 30 and 60 Mg ha^{-1}) and Plant Survival (alive and dead). Tables correspond to the air-dried sewage sludge (up) and composted sewage sludge (down). In bold, significant differences (p<0.100). Different letters correspond to significant differences in the Treatment Factor

Dry Sewage Sludge

	Survival (%)	Basal Diameter (mm)	Pine Biomass (g)	Litter ($g\ m^{-2}$)		Natural Vegetation ($g\ m^{-2}$)		Roots ($mg\ cm^{-3}$)		Soil Organic Matter (%)	
				Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
E0	42.3ab	5.1 ± 0.9a	29.8 ± 8.5a	32.3 ± 5.9	32.6 ± 21.4	8.2 ± 8.1	60.9 ± 50.7	2.46 ± 0.33	1.50 ± 0.39	4.25 ± 0.19	4.80 ± 0.40a
EL15	46.7a	8.7 ± 0.6ab	69.2 ± 8.4b	64.7 ± 26.7	90.8 ± 45.0	18.2 ± 6.2	22.6 ± 14.1	3.27 ± 0.27	2.96 ± 0.47	5.66 ± 0.64	5.03 ± 0.40ab
EL30	43.3a	10.2 ± 1.3b	99.9 ± 21.5b	106.8 ± 33.5	46.7 ± 12.0	36.2 ± 11.2	34.1 ± 17.0	3.23 ± 1.15	1.84 ± 0.39	4.68 ± 0.11	4.11 ± 0.48a
EL60	12.9b	6.4 ± 1.3ab	41.9 ± 15.2ab	32.7 ± 23.4	116.0 ± 49.7	12.0 ± 9.4	8.0 ± 5.5	3.03 ± 0.17	4.75 ± 1.04	4.80 ± 0.77	6.13 ± 0.68b
Treatment	**χ^2=9.549 p=0.049**	**F=3.950 p=0.016**	**F=4.613 p=0.008**	F=0.669 p=0.579		F=1.241 p=0.314		F=1.690 p=0.194		**F=2.664 p=0.069**	
Plant Survival	--	--	--	F=0.210 p=0.651		F=0.110 p=0.743		F=0.161 p=0.692		F=0.252 p=0.620	

Composted Sewage Sludge

	Survival (%)	Basal Diameter (mm)	Pine Biomass (g)	Litter ($g\ m^{-2}$)		Natural Vegetation ($g\ m^{-2}$)		Roots ($mg\ cm^{-3}$)		Soil Organic Matter (%)	
				Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
E0	42.3	5.1 ± 0.9a	29.8 ± 8.5a	32.3 ± 5.9	32.6 ± 21.4	8.2 ± 8.1	60.9 ± 50.7	2.46 ± 0.33	1.50 ± 0.39	4.25 ± 0.19	4.80 ± 0.40
EC15	45.2	8.1 ± 0.8ab	62.1 ± 10.3b	120.0 ± 69.2	97.9 ± 78.5	16.3 ± 10.3	154.2 ± 94.4	4.09 ± 0.79	2.57 ± 1.03	5.18 ± 0.34	4.93 ± 1.26
EC30	37.9	9.0 ± 1.1b	76.0 ± 18.1b	50.3 ± 12.9	227.5 ± 120.2	33.6 ± 14.3	29.6 ± 17.0	3.97 ± 0.59	3.16 ± 0.55	5.40 ± 0.19	5.38 ± 0.61
EC60	25.9	6.4 ± 1.4ab	41.4 ± 15.6ab	220.4 ± 200.8	59.0 ± 19.0	39.0 ± 32.6	1.2 ± 0.6	4.18 ± 1.60	1.97 ± 0.46	5.60 ± 1.15	6.39 ± 0.63
Treatment	χ^2=3.817 p=0.431	**F=3.014 p=0.051**	**F=3.766 p=0.025**	F=0.859 p=0.475		F=0.813 p=0.498		F=1.486 p=0.241		F=1.329* p=0.286	
Plant Survival	--	--	--	F=0.074 p=0.788		F=0.009 p=0.925		**F=5.957 p=0.022**		F=0.271 p=0.607	

*: variance homogeneity was not achieved.

Different letters in the same column indicate significant differences between treatments, irrespective of whether the planted pine was dead or alive.