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Soil CO_2 efflux as early response assessment for remediation of diesel polluted soils

Las emisiones de CO_2 del suelo como evaluación temprana de la remediación de suelos contaminados con diésel

As emissões de CO_2 do solo como forma precoce de avaliação da resposta à remediação de solos contaminados com diesel

AUTHORS

Fernández Alonso M.J.[@,1]
mj.fernandez@upm.es

Ortiz Oñate C.[1,2]

Gómez Fernández L.[1]

Rubio Sánchez A.[1]

[@] Corresponding Author

[1] Department of Systems and Natural Resources, School of Forestry Engineering and Natural Resources, Technical University of Madrid. Ciudad Universitaria s/n. 28040 Madrid, Spain.

[2] Department of Agricultural Production, School of Agricultural Engineering, Technical University of Madrid. Ciudad Universitaria s/n. 28040 Madrid, Spain.

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ABSTRACT

Soil contamination by petroleum hydrocarbons constitutes a considerable environmental risk due to their toxicity. In recent decades, several biological and chemical technologies have been developed for remediating *in situ* soils and waters affected by leakages of diesel fuel. The aim of this study is to assess the soil CO_2 efflux as an early measuring tool of the effectiveness of these remediation treatments applied *in situ* on diesel polluted soils. The study site was located in a tidal salt marsh ecosystem in the Cádiz Bay, where two zones were distinguished according to the level of diesel pollutant (high-polluted and low-polluted areas). In the high-polluted area, three remediation technologies (phytoremediation, bioremediation, and chemical oxidation) were applied individually as well as in combination in order to identify synergies that improve the decontamination performance. The specific objectives of the study were (1) to determine soil CO_2 efflux in a diesel polluted tidal salt marsh under a Mediterranean climate; (2) to examine the relationships between soil moisture content, soil temperature and soil CO_2 efflux; (3) to test whether the different remediation treatments promote an early response in soil CO_2 efflux. The initial results showed a positive correlation between soil temperature fluctuations and soil CO_2 efflux in the low-polluted area of the marsh, but no significant relationships were detected in the high-polluted area. On average, remediation treatments lead to greater soil CO_2 efflux rates (81.3 and 294.8 mg CO_2-C m^{-2} h^{-1} before and after treatment implementations, respectively). Of all the remediation treatments, only those plots in which pure biological treatments were employed (phytobarrier, phytoremediation and bioremediation) displayed a clear early response in soil CO_2 efflux.

RESUMEN

La contaminación del suelo por hidrocarburos de petróleo constituye un grave riesgo ambiental debido a su toxicidad. Se han desarrollado a lo largo de las últimas décadas diversas tecnologías biológicas y químicas para remediar in situ *suelos y aguas afectadas por vertidos de petróleo. El objetivo de este estudio es evaluar el flujo de emisión de CO_2 del suelo como medida temprana de la efectividad de estos tratamientos de remediación* in situ *de suelos contaminados por hidrocarburos de petróleo. El área de trabajo se localiza en un ecosistema de marisma salina en la Bahía de Cádiz, donde se distinguieron dos zonas atendiendo a su nivel de contaminación por diésel (zona altamente contaminada y zona con baja contaminación). En la zona altamente contaminada se aplicaron tres tecnologías de remediación* in situ *(fitorremediación, biorremediación y oxidación química) de forma individual y combinada, para buscar sinergias que mejoren el rendimiento de la descontaminación. Los objetivos específicos del estudio fueron (1) determinar el flujo de emisión de CO_2 del suelo en un ecosistema de marisma salina contaminada por diésel, bajo*

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clima Mediterráneo; (2) examinar las relaciones entre la temperatura y el contenido de humedad del suelo con los flujos de CO_2 del suelo; (3) evaluar si las diferentes tecnologías remediadoras fomentan una respuesta temprana en los flujos de CO_2 del suelo. Los primeros resultados mostraron una correlación positiva entre las fluctuaciones de la temperatura del suelo y el flujo de CO_2 en la zona menos contaminada de la marisma, pero no se detectaron relaciones significativas en el área altamente contaminada. De media, los tratamientos de remediación produjeron mayores flujos de CO_2 del suelo (81,3 y 294,8 mg CO_2-C m^{-2} h^{-1} antes y después de la implementación de los tratamientos, respectivamente). De todos los tratamientos de remediación, sólo las parcelas bajo tratamiento biológico puro mostraron una respuesta temprana del flujo de CO_2 del suelo (fitobarrera, fitorremediación y biorremediación).

RESUMO

A contaminação do solo por hidrocarbonetos de petróleo constitui um sério risco ambiental devido à sua toxicidade. Durante as última décadas foram desenvolvidas diferentes tecnologias biológicas e químicas para remediar in situ *o solo e as água afetadas por derramamentos de petróleo. O objetivo deste estudo é avaliar o fluxo de emissão de CO_2 do solo como medida precoce da eficácia destes tratamentos de remediação* in situ *de solos contaminados com hidrocarbonetos. A área de trabalho está localizada num ecossistema de pântanos de água salgada na Baía de Cádiz, onde se diferenciam duas áreas atendendo ao seu nível de poluição com diesel (zona altamente contaminada e uma zona de baixa poluição). Na área altamente contaminada foram aplicadas três tecnologias de remediação* in situ *(oxidação química, biorremediação e fitorremediação) de forma individual e combinada, para encontrar sinergias por forma a melhorar o rendimento da descontaminação. Os objectivos específicos do estudo foram (1) determinar o fluxo de emissão de CO_2 do solo num ecossistema de pântanos de água salgada contaminado por diesel, sob clima mediterrânico; (2) estabelecer a relação entre a temperatura e o teor de humidade do solo com os fluxos de CO_2 do solo; (3) avaliar se as diferentes tecnologias de remediação promovem uma resposta precoce dos fluxos de CO_2 do solo. Os primeiros resultados mostraram uma correlação positiva entre as flutuações da temperatura do solo e o fluxo de CO_2 na área menos poluída do pântano, mas não se detetaram relações significativas na área altamente contaminada. Em média, os tratamentos de remediação produziram maiores fluxos de CO_2 de solo (81,3 e 294,8 mg CO_2-C m^{-2} h^{-1} antes e após a aplicação dos tratamentos, respetivamente). De todos os tratamentos de remediação, somente as parcelas sob tratamento biológico puro apresentaram uma resposta precoce do fluxo de CO_2 do solo (fitobarreira, fitorremediação e biorremediação).*

KEY WORDS
Bioremediation, phytoremediation, *in situ* chemical oxidation (ISCO), total petroleum hydrocarbons (TPH), soil respiration

PALABRAS CLAVE
Biorremediación, fitorremediación, oxidación química *in situ*, hidrocarburos totales de petróleo, respiración del suelo

PALAVRAS-CHAVE
Biorremediação, fitorremediação, oxidação química *in situ*, hidrocarbonetos totales de petróleo, respiração do solo

1. Introduction

Soil and groundwater pollution by petroleum hydrocarbons is an increasing problem due to the large-scale use of fossil fuels. Various hydrocarbon compounds may be leaking into the soil as a result of transport, storage and refining activities, as well as accidental spills (Gallego et al. 2001). The presence of total petroleum hydrocarbons (TPH) in soils is a problem that has caused concern worldwide because it poses a huge threat to human health and natural ecosystems (Chen et al. 2015; Li et al. 2015). The fate of these compounds in the environment is often leaching into groundwater, dissolution in pore water, adsorption onto or absorption into soil particles, present as a separate phase as well as being biodegraded (Lai et al. 2009; Huguenot et al. 2015). According to the Spanish National Inventory, the potential number of contaminated sites in Spain is 4 532 (Tarazona et al. 2005), 34.3% of which have reported petroleum hydrocarbons as the pollution source (Majone et al. 2015). Due to their harmful effects and toxicity, national regulations have been established with regard to maximum admissible values according to the soil quality standards (Pinedo et al. 2013). Spain regulates in accordance with RD 9/2005, a generic reference level (NGR from its Spanish acronym) in soils of 50 mg TPH kg^{-1} dry weight, which establishes the legal threshold at which a site-specific risk assessment must be performed (Presidency Ministry 2005). In several European countries, a second threshold is used to define a soil as contaminated, so that when an unacceptably high concentration of pollutants is reached a remediation procedure should be performed. For example, in the Netherlands, an intervention value (IV) of 5 000 mg TPH kg^{-1} dry weight for soil and sediment is adopted, which is 100 times greater than the NGR in Spain (Lijzen et al. 2001; Presidency Ministry 2005).

Once a soil has been declared contaminated, it is important to choose the most appropriate remediation technology in each case, which is not always easy. At present, efforts to remediate TPH polluted sites are strongly oriented towards the use of *in situ* treatment methods (Huguenot et al. 2015). These approaches have the advantages of being more cost-effective, logistically efficient and more environmentally friendly than *ex situ* processes (Camenzuli and Freidman 2015). *In situ* biological remediation technologies use the natural degradative ability of plants (phytoremediation) or microorganisms (bioremediation) to transform contaminants into less toxic compounds (Lai et al. 2009). Biological technologies are considered green, efficient and low-cost remediation approaches but require overall longer remediation times and are only advantageous when applied to diffuse sources of contamination (Li et al. 2015; Beames et al. 2015). In contrast, *in situ* chemical oxidation (ISCO) focuses on the injection of a strong oxidizing agent (Fenton's reagent) in order to directly oxidize the hydrocarbons into less harmful chemical species, ideally water and carbon dioxide (CO_2) (Cadotte et al. 2007; Lemming et al. 2012). In comparison to biological remediation technologies, ISCO is both cost-effective and relatively fast, therefore can potentially be used to treat large amounts of polluted soil, although risks as well as waste production can be greater (Huguenot et al. 2015; Li et al. 2015). Hence, there is a need to evaluate the diesel remediation performance of these *in situ* technologies via an early response assessment.

Terrestrial soil CO_2 efflux results from autotrophic (living roots and mycorrhizae) and heterotrophic (decomposer organisms) activity (Högberg et al. 2001) and is more than ten times greater the anthropogenic flows resulting from fossil fuel combustion (Raich and Tufekciogul 2000). It is widely accepted that to understand CO_2 efflux dynamics in ecosystems, soil temperature (*T*) and soil moisture (*M*) are two important parameters to consider (Qi and Xu 2001). In diesel polluted soils, soil CO_2 efflux resulting from oxidation and biodegradation processes in the TPH remediation operations is added to the natural soil CO_2 efflux. In this study, we conducted an experimental trial in a diesel polluted soil in which ISCO, phytoremediation and bioremediation treatments were employed. To evaluate the performance of each treatment the soil CO_2 efflux was analyzed, taking into account environmental variables –soil *T* and *M*– and the TPH level.

The hypotheses considered for this study were: (a) soil CO_2 efflux responds to soil *T* and soil *M*; (b) soil CO_2 efflux is related to TPH level; (c) soil CO_2 efflux reflects an early response to diesel remediation treatments. Three specific objectives were established: (1) to determine soil CO_2 efflux in a diesel polluted tidal salt marsh under a Mediterranean climate; (2) to examine the relationships of soil *T* and *M* with soil CO_2 efflux; (3) to test whether the different remediation treatments promote an early response in soil CO_2 efflux.

2. Materials and methods

2.1. Location and site description

The study site was located on the Atlantic coast of Southern Spain (municipality of San Fernando, province of Cádiz: Datum ETRS89: 751698 E, 4042756 N, Zone 29S; 1.5 m.a.s.l.) (**Figure 1**) at *La Clica*, an area belonging to the Spanish Army where there is a working fuel supply terminal for vessels. The experimental area comprises 36 100 m^2 of diesel polluted soil caused by accidental leakages from diesel storage tanks and transportation operations over a period of at least 20 years.

The site is characterized by a Mediterranean-oceanic climate with warm, dry summers (4 to 5 month dry period), and a mild, humid, frost-free winter. The mean annual temperature is 18.6 ºC, and the mean annual precipitation is 523 mm, mostly distributed over the autumn and winter months. Soils are defined as Entisols (Soil Survey Staff 2014) formed over salt marsh deposits, with an aquic moisture regime caused by a period of permanent water saturation due to seasonal variability in the water table.

An initial characterization of the study site was performed by digging 25 soil pits for the complete soil profile, at regular intervals over the area affected by the diesel leakage (**Figure 1**). Composite soil samples from every genetic horizon were collected for measuring pH, electrical conductivity, texture, inorganic carbon (using the Bernard calcimeter method), total carbon and total nitrogen (N) (Truman CN analyzer, Leco Corp, Castle Hill, NSW Australia) and TPH (Jiménez et al. 2014). The soils have high salinity, alkaline pH and a clay-loam texture that gradually increases in clay content with depth until it reaches a horizon of compact sediment slurry. The organic carbon (C) concentration in the first 10 cm ranges from one to 28 mg g^{-1} dry soil and the total N concentration ranges from 0.3 to 1.08 mg g^{-1}. Soil TPH was irregularly distributed in both vertical and horizontal dimensions, reaching maximum values at the sediment slurry horizon. The general TPH values are greater than both NGR and IV soil quality standards, and therefore the soils at the study site should be decontaminated.

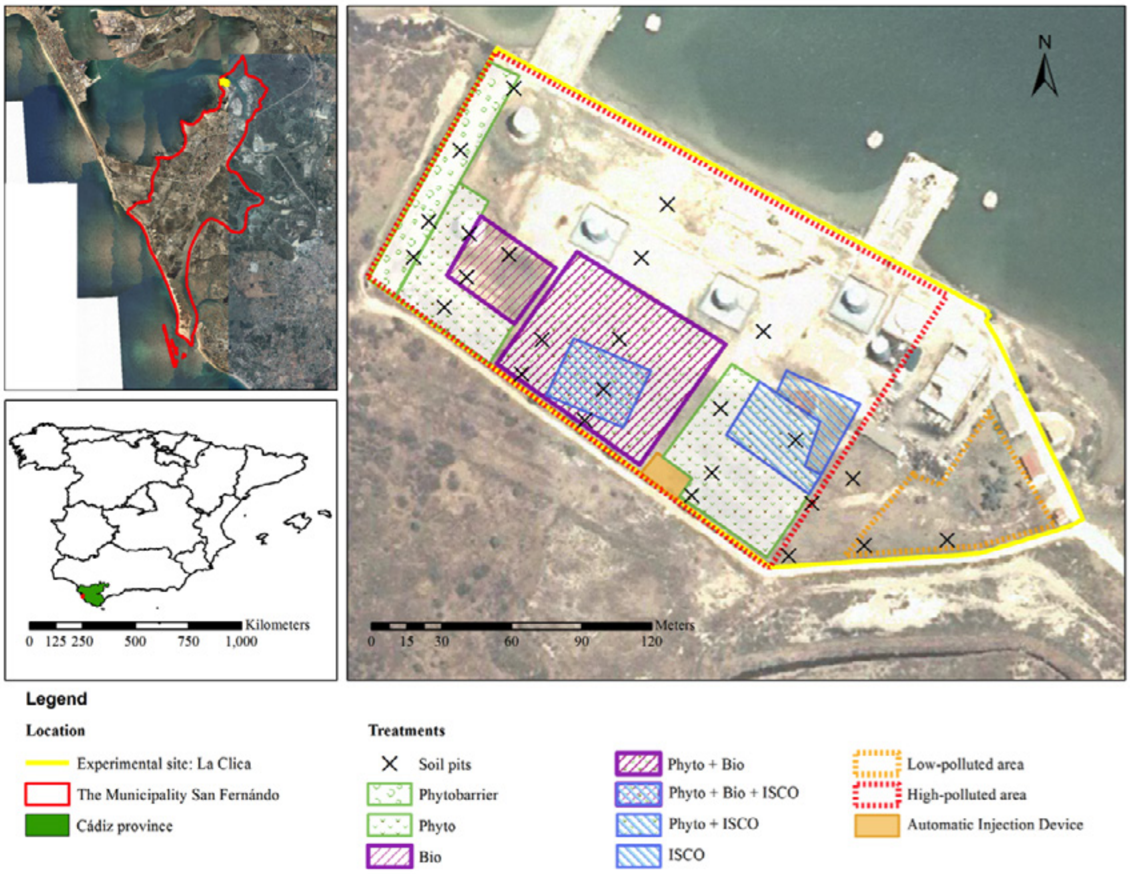

Figure 1. (Left) Location of experimental study site at *La Clica* (San Fernando, Cádiz province, Spain). (Right) Delimitation of the treatment zones within the study site. Remediation treatments are denoted by: Phyto - phytoremediation, Bio - bioremediation and ISCO - chemical oxidation.

Taking the distribution of TPH values into consideration, the study site was divided into two main zones, a low-polluted area and a high-polluted area (126.7 ± 87.1 mg kg^{-1} and 965.8 ± 833.9 mg kg^{-1} dry soil in the top 40 cm of soil) (**Figure 1**). In the high-polluted area, a containment measure and three different in situ remediation technologies were implemented (see next section for details), while the low-polluted area was used as a control. Levels of TPH pollution in the top 40 cm of soils were estimated by averaging the values obtained from the analysis of samples collected in the 25 soil pits (**Figure 2**).

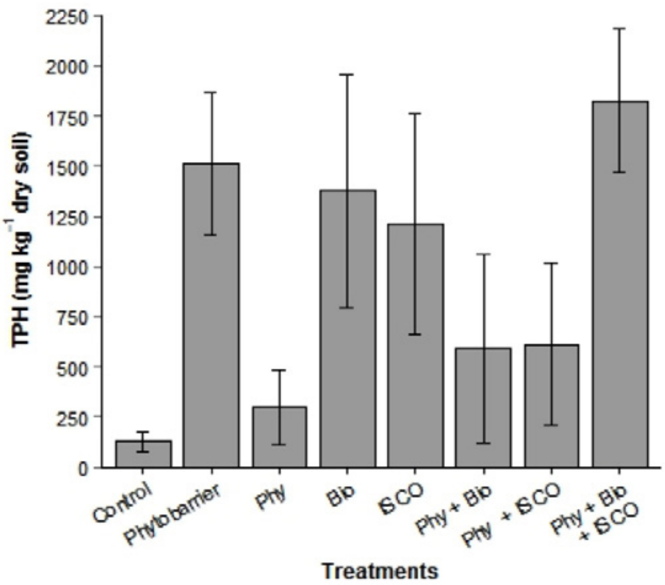

Figure 2. Initial mean pollution levels in treatment plots for the first 40 cm of soil. Vertical bars denote ± SE (n=3). Remediation treatments are denoted by: Phyto - phytoremediation, Bio - bioremediation and ISCO - chemical oxidation.

2.2. Remediation treatments

Three remediation technologies were selected for *in situ* decontamination of the high-polluted area: phytoremediation, bioremediation and ISCO. In addition, different combinations of these three technologies were also employed in an attempt to identify possible synergies that could improve the remediation performance. Furthermore, a phytobarrier was established as a containment measure. To estimate the decontamination performance of the different technologies and their combinations, the low-polluted area was used as a control in the analysis. Thus, eight different scenarios were explored in this study: phytobarrier, phytoremediation, bioremediation, ISCO, phyto + bioremediation, phytoremediation + ISCO, phyto + bioremediation + ISCO and the low-polluted area. The latter was used for purposes of comparison and no treatment was applied in this area.

Phytoremediation was established in most of the high-polluted area. In total, 2 200 trees from 300 cm^3 pots and with a height of 1-1.2 m were planted manually using a regular planting pattern of 2 x 3 m following a subsoil operation in March, 2014. Plant species were selected according to their ability to remediate as well as their adaptability to other site-specific factors: *Populus nigra L.*, *P. alba L.*, *Tamarix gallica L.*, *Pistacia lentiscus L.* and *Salix purpurea L.* The phytobarrier treatment using *T. gallica L*. was established on the northwestern edge of the site with a regular planting pattern (2 x 2 m) to prevent the uncontrolled flow of pollutants outside the affected area. For these two types of treatment an initial slow release fertilizer (18-6-8(2MgO) + ME) was added to ensure plantation success in accordance with supplier recommendations. Furthermore, daily drip irrigation (ranging from 0.2 to 1 L per plant and day, according to the hydric requirements of each species) and periodical manual weed control were applied.

In the ISCO treatment, 44 700 L of hydrogen peroxide (solution 10%) distributed over five injection rounds was applied. The oxidant was pumped down through 50 polyvinylchloride (PVC) injection wells (see Reino et al. 2014 for details). To increase the radius of influence, a catalyst solution (chelated iron) was also injected separately. The oxidant dosage and sequence of the injections were adjusted individually for each treatment, according to the direction of the water flow and the amount of residual hydrocarbons in each period.

In the Bioremediation treatment, mechanical ploughing to a depth of 40 cm was carried out to promote aeration and drainage as well as to prevent soil compaction. In a second step, 1 000 L of the surfactant Surfsoil® (Soilutions Medio Ambiente CB, Madrid, Spain), and 1 000 L of the fertilizer S-200 (IEP Europe S.L., Madrid, Spain) diluted in water were applied twice during 2015 (January and May) using sprinkler irrigation. These treatments stimulate aerobic microbial activity in the soil (optimal conditions of moisture, nutrients and aeration) leading to a higher rate of biodegradation of the soil-adsorbed TPH by the indigenous soil microbial community.

2.3. Soil CO_2 field measurements

Soil CO_2 efflux was monitored seasonally between 10.00 a.m. and 18.00 p.m., avoiding extreme temperatures, by using a flow-through non-steady-state system (Savage and Davidson 2003). Three randomly located points per treatment were monitored throughout the whole study period. A total of 27 PVC rings (3.2 dm^2 surface soil) were inserted into the soil to a depth of 2 cm. Manual measurements were carried out by placing a PVC respiratory chamber (4.04 L volume) over the rings and coupling them to a portable infrared gas analyser (IRGA) WMA-4 (PP Systems, Hertfordshire, United Kingdom). This creates a closed gas exchange system with air circulating between the chamber and the IRGA at a rate of 0.5 l min^{-1}. The change in CO_2 concentration within the respiratory chamber was recorded over a time period of five minutes with measurements every 30 seconds. Air temperature, soil *M* and soil *T* were also monitored in close proximity to each soil respiration chamber at the same time as the soil CO_2 efflux was measured. Soil *M* at a depth of 10 cm was measured by time domain reflectometry (Field Scout TDR 100, Spectrum Technologies Inc, USA). Soil and air *T* were monitored using a Termistor Vertix 5989M probe (Herter Instruments, Barcelona).

The soil-surface CO_2 efflux rate was calculated as the linear increase in the gas concentration in the chamber headspace over the fixed time that it remains closed, and corrected for atmospheric pressure and chamber air temperature. Based on the recommendations of Parkin et al. (2012), the estimated minimum detectable soil-surface CO_2 efflux during the experiment ranged from 6.2 to 7.1 mg CO_2-C m^{-2} h^{-1}.

2.4. Statistical analyses

Non-linear regression analyses were performed in order to study the effects of environmental variables, soil *T* (**Eq. 1**) and *M* (**Eq. 2**), on soil CO_2 efflux. The following first-order exponential functions were fitted according to Rayment and Jarvis (2000), Fang and Moncrieff (2001) and Qi and Xu (2001).

(Eq.1)	$Rs\ (T) = \alpha \cdot e^{\beta T}$
(Eq.2)	$Rs\ (M) = \gamma \cdot e^{\delta M}$

Where *Rs* is the soil CO_2 efflux rate (mg CO_2-C m^2 h^{-1}); *T* is the soil temperature (ºC); *M* is the volumetric soil water content (cm^3 water · 100 cm^{-3} soil, expressed as %); α and γ are the efflux rate at 0 ºC and 0% water content (i.e. basal rate); and β and δ are parameters describing the soil *T* and *M* sensitivity, respectively.

In the low-polluted area, the database used to fit both curves comprised all measurements registered from January 2014 to July 2015. In the high-polluted area, two data subsets were needed in order to differentiate base line patterns (data collected in early spring 2014) denoted by a subscript '0' (Rs_0, T_0, M_0), and the response after the implementation of remediation treatments (data collected in summer 2015), denoted by a subscript '1' (Rs_1, T_1, M_1).

A new variable (ΔRs) was included in the statistical analyses to account for the early response of soil CO_2 efflux, and calculated as follows (**Eq. 3**),

(Eq.3)	$\Delta Rs = Rs_1 - Rs_0$

An analysis of covariance (ANCOVA) was first conducted to test the effects of TPH pollution levels on ΔRs among remediation treatments. The ΔRs data were log-transformed to satisfy the normality and homoscedasticity assumptions of ANCOVA; no transformation was necessary for TPH. Further one-way analysis of variance (one-way ANOVA) and pair-wise post-hoc Bonferroni corrected t-test were conducted to ascertain differences among remediation treatments in ΔRs.

All the analyses were conducted in R (R Development Core Team 2014), and its packages 'lmtest' (Zeileis and Hothorn 2002) and 'car' (Fox and Weisber 2011). The significance level was 0.05 for all the analyses performed.

3. Results and discussion

3.1. Relationships between soil CO_2 efflux, soil temperature and soil moisture in the low-polluted area

The dependence of *Rs* on the soil *T* and *M* in low-polluted areas was studied separately from the high-polluted area over a 19 month period. The measurements of *Rs* presented in this study (25.5-299.1 mg CO_2-C m^2 h^{-1}) fall within the range of 7.5-380.3 mg CO_2-C m^2 h^{-1} presented by Frankignoulle (1998) and Roehm (2005) for other tidal salt marshes and coastal wetlands. A first-order exponential regression was used to explain the relationship between *Rs* and both environmental variables, soil *T* and *M*, individually. On an annual scale, the univariate regression between *Rs* and soil *T* (**Figure 3a**) showed a positive and significant correlation, but there was no significant correlation between *Rs* and soil *M* (**Figure 3b**). The soil *T* exhibited a typical Mediterranean seasonal dynamic –the highest air *T* in the driest period–, but soil *M* did not follow this pattern since the highest values did not always correspond to the wet seasons (autumn and winter). Due to the location of the study site in Cádiz Bay, a specific periodic hydrological condition existed, *i.e.* tidal fluctuations, which influence the level of the water table and waterlogging. In this regard, Guo et al. (2009) reported that tidal fluctuations do not co-vary with soil *T* since they have a semidiurnal pattern over a 15 day period. This could explain why *Rs* at *La Clica* was not driven by the effect of soil *M*, in contrast to other studies conducted in Mediterranean areas (Almagro et al. 2009). In addition, the influence of tides on *Rs* is not only due to the groundwater level but also to other relevant chemical properties of the water such as pH, redox potential, nutrient content, salinity or dissolved inorganic carbon (Guo et al. 2009; Cai 2011). All these major factors may be confounding the response of *Rs* under field conditions. Further research should compare the effect of variations in soil *M* on *Rs* on a smaller timescale (e.g. monthly instead of annually) to identify possible correlations between both variables.

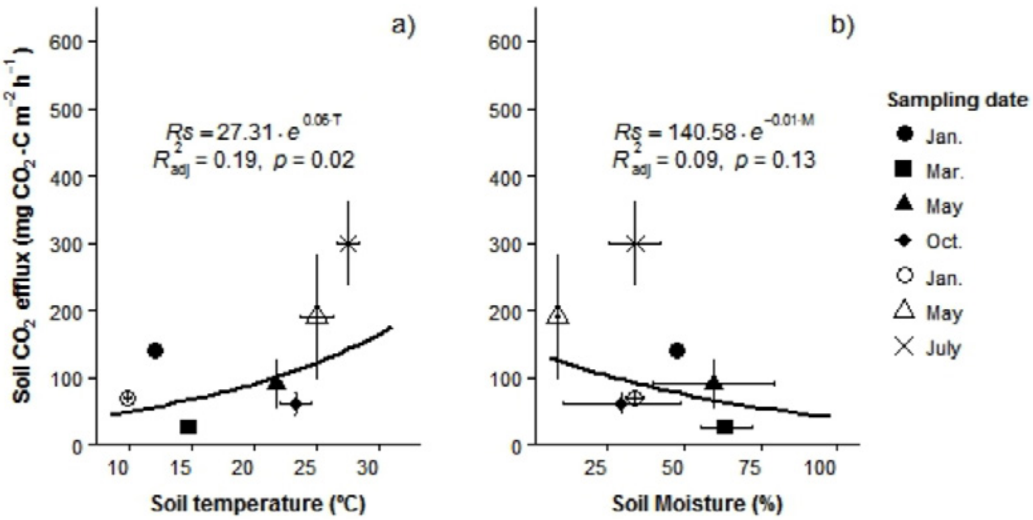

Figure 3. First-order exponentially-fitted models between soil-surface CO_2 efflux (*Rs*) registered in the low-polluted area from January 2014 to July 2015 and a) soil temperature (*T*), or b) soil moisture (*M*). Each data represents the mean value of three respiratory chambers. Error bars are the standard error for the estimated mean. Solid shapes refer to 2014 field campaigns, while empty shapes refer to 2015.

3.2. Relationships between soil CO_2 efflux, soil temperature and soil moisture in the high-polluted area: comparison between non-remediation and implementation of remediation treatments

The relationships between *Rs* and both environmental variables, soil *T* and soil *M*, were also addressed for high-polluted area. To assess the effect of diesel pollution, a base line for soil CO_2 efflux (Rs_0) was established in 2014. Data from the 24 respiratory chambers placed in the treatments were used as replicates to fit the curves. Before the implementation of remediation treatments the mean Rs_0 was 81.3 mg CO_2-C m^{-2} h^{-1} (± 36.1 SE). Neither soil T_0 nor M_0 showed significant relationships with soil Rs_0 (**Figure 4**); the lack of relationship between Rs_0 and soil T_0 contrasts with the response found in the low-polluted area. This fact could be a consequence of factors that limit the microbial activity in the high-polluted area, linked to the physicochemical properties of the polluted soil and to the degree and characteristics of the diesel spills (Fuentes et al. 2014). On the one hand, the excess C source due to the high TPH concentration in these soils does not mean an increase in microbial activity due to the low levels of other essential nutrients, especially N and phosphorous (P), which are necessary for an optimal organic decay process (Chang et al. 2010). Furthermore, the insufficient oxygen availability during periods of rising water levels at *La Clica* could contribute to constrain the natural attenuation, as an electron donor is needed for the aerobic hydrocarbon degradation process (Shahi et al. 2016). On the other hand, due to the diesel degradation dynamics in natural environments, most of the residual hydrocarbons still present in this aged diesel contaminated soil may have high molecular weights and can therefore be considered recalcitrant, if their state of adsorption to the soil matrix is taken into account (Fuentes et al. 2014). In long-term diesel leakages, the more readily degradable components –soluble hydrocarbons with low molecular weight– are already volatilized or metabolized by microorganisms (Bento et al. 2005; Ron and Rosenberg 2014). As a consequence of these two limiting issues –edaphic conditions and the chemical nature of the pollutant– it is highly likely that the growth and activity of the diesel degrading microorganisms in the high-polluted area were slowed, hence Rs_0 did not respond to an increase in soil T_0 as stated for soil *T* in the low-polluted area.

The soil CO_2 efflux measured in summer 2015 (Rs_1) was used to assess the response to the implementation of remediation treatments in the high-polluted area. The mean efflux rate calculated for Rs_1 was 294.8 mg CO_2-C m^{-2} h^{-1} (± 41.0 SE), 3.6 times higher than the soil CO_2 efflux registered in the base-line scenario. This result would appear to confirm that the remediation treatments were (1) improving the physicochemical properties of the diesel polluted soil, as well as (2) promoting the degradation of TPH pollutants. Mater et al. (2007) reported that both the degree of mineralization and the biodegradability of the contaminants were enhanced by Fenton's reagent in an experiment carried out in a closed reactor with a petroleum-contaminated soil. They also found that the CO_2 evolved during the trial was an operative approach to evaluating the effectiveness of the oxidation of organic compounds. Despite the fact that a fraction of the injected oxidant in the ISCO treatment may be consumed by organic matter oxidation processes, termed natural oxidant demand (Lemming et al. 2012), the initial results from laboratory assays using microcosms with soil samples from *La Clica* (Díaz-Puente et al. 2015) showed a TPH removal efficiency of between 73 and 80%. In this study, samples collected *in situ* after the injection of oxidant during field monitoring campaigns, showed an increase in microbial activity and population counts compared to samples collected before the injection round. The higher Rs_1 in bioremediation treatment plots might also be due to an increase in the TPH degradation rate by indigenous microorganisms as a consequence of restoring the C-TPH:N:P nutrient balance through fertilizer amendments. Gao et al. (2014) found that the addition of N stimulated TPH degradation significantly in the initial 30 days of the remediation treatment of oil contaminated saline soil but that after this period, the accumulation of recalcitrant compounds inhibited the microbial degradation. In our experiment, the addition of biosurfactants may enhance the desorption and solubilisation of TPH in the soil matrix, thus facilitating their assimilation by microorganisms (Lai et al. 2009) and hence maintaining a high Rs_1 over time. Finally, the root growth in the phytobarrier and phytoremediation treatments increase nutrient supply in the form of exudates, the exchange of gases and the bioavailability of TPH (Khan et al. 2013), implying an improvement in soil physicochemical and biological properties. The improvement of soil properties in the rhizosphere might support an increase in the population and activity of TPH degrading microbes, and consequently may lead to higher Rs_1 due to the removal of pollutant.

In the high-polluted area, the relationships of Rs_1 with soil T_1 and soil M_1 were non-significant (**Figure 4**). The implementation of remediation treatments might affect the functioning of the soil so severely that Rs_1 response is not driven by environmental variables and furthermore its values are maximized. At the end of the

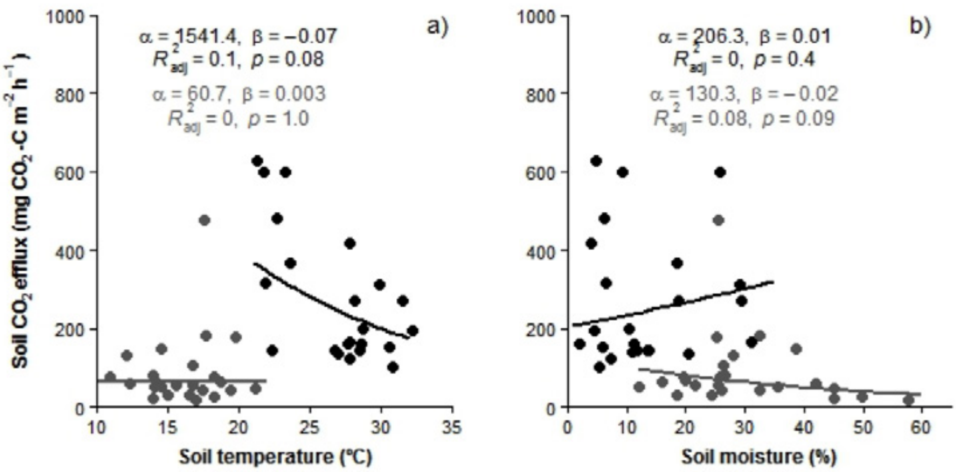

Figure 4. Fitted relationships between mean soil CO_2 efflux and a) soil temperature or b) moisture in the high-polluted area (n = 24). Grey dots refer to base line soil CO_2 efflux measured in summer 2014 (Rs_0) and black dots refer to mean soil CO_2 efflux in response to the implementation of all remediation treatments measured in the summer 2015 (Rs_1).'

remediation process, the recovery of soil properties and the removal of pollutants could re-establish the dependence of *Rs* on soil *T* as occurred in the low-polluted area.

3.3. Early response soil CO_2 efflux for remediation treatments

The effect of each treatment was evaluated by the difference in *Rs* between the base line and the early response scenarios (ΔRs, **Eq.3**). Due to the irregular level of pollutant in the study area (**Figure 2**), it was necessary to assess the interactions of TPH concentrations with treatments; however, the ANCOVA analysis was non-significant ($p > 0.05$). Furthermore, single treatment effects were analysed using one-way ANOVA and were found to be relevant for explaining ΔRs ($p < 0.05$); although post-hoc tests only revealed significant differences in the efflux rates of individually-implemented biological treatments (**Figure 5**). Thus, the ΔRs in the bioremediation treatment was significantly greater than those corresponding to the phytobarrier and phytoremediation treatments. These marked differences in ΔRs may be due to a greater TPH degradation efficiency of bioremediation treatments through an improvement in soil conditions for microbial activity. As stated above, the addition of surfactant promotes TPH mobilisation, which together with improved nutrient status of the soil through fertilization, may increase the natural attenuation rate. This is consistent with studies that point to N as the most important limiting factor to biological degradation of TPH in soils (Fuentes et al. 2014; Gao et al. 2014; Ron and Rosenberg 2014). Furthermore, the indigenous microbial communities exposed to diesel pollutant over a long period of time have become adapted, and are able to quickly respond to hydrocarbons and exhibit higher biodegradation rates (Chirwa and Bezza 2015). Hence, a higher ΔRs might be expected as an early response to the bioremediation process. In contrast, the improvement in soil properties through plantation is a slower process that entails the development of a plant-microbe interaction in the rhizosphere, thus the early response to pollutant degradation in phytobarrier and phytoremediation treatments is lower than for bioremediation. Gao et al. (2014) reported an amelioration of soil properties through phytoremediation, but they only identified a significant positive effect on TPH degradation when this treatment was coupled with other remediation techniques, as found in the present study (**Figure 5**). The contrasting approaches of biological and chemical

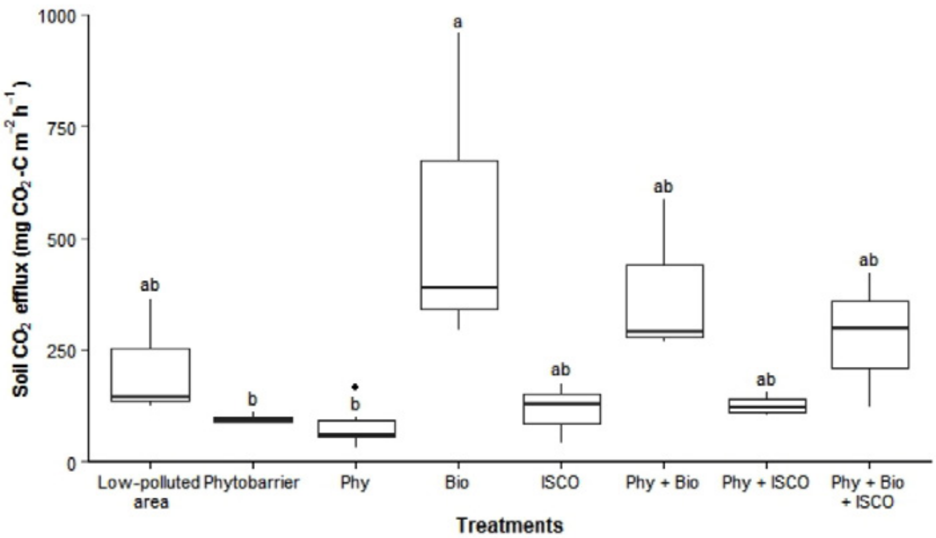

Figure 5. Tukey boxplot for the difference in soil CO_2 efflux registered prior to and after the remediation development (ΔRs) for each treatment plot (n = 3). Different lower case letters indicate significant differences among remediation treatments after one-way ANOVA followed by pair-wise Bonferroni corrected t-test ($p < 0.05$). Remediation treatments are denoted by: Phyto - phytoremediation, Bio - bioremediation and ISCO - chemical oxidation.

remediation techniques may underlie the lack of early response under the ISCO treatment, since the injection of an oxidant immediately degrades the diesel pollutant producing a short *Rs* peak, while biodegradation through the phyto- and bioremediation treatments is a more progressive and continuous process (**Figure 3**).

If the early response to treatments observed in this study is supported by future results, the conclusion may be drawn that the effectiveness of different treatments can be assessed during the initial stages of the remediation process by measuring the biological activity in the soil through the amount of soil CO_2 efflux evolved from the oxidation of TPH pollutants.

4. Conclusions

Greater mean efflux rates were observed after the implementation of the remediation techniques in a diesel polluted area of the tidal salt marsh in Cádiz Bay. The results from all remediation treatments point to a clear early response in soil CO_2 efflux in individually implemented biological treatment plots (phytobarrier, phytoremediation and bioremediation). Where the influence of high levels of pollutants was absent, there was a positive correlation between soil temperature fluctuation and soil CO_2 efflux, while soil moisture was not relevant; the greatest soil respiration rates in this particular ecosystem occurred in summer. Before implementing the remediation treatments in the high-polluted area, there was no evidence of temperature or moisture affecting soil respiration. Similarly, there were no relationships between soil CO_2 efflux and both soil environmental variables during the initial months after the remediation treatment began in high-polluted areas. Long-term monitoring of the remediation processes could help to confirm whether these early responses are consistent over time thereby validating the use of early soil CO_2 efflux response as a tool for measuring the effectiveness of remediation treatments in diesel polluted soils.

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