



A full-scale bioremediation study of diesel fuel-contaminated soil: the effect of plant species and soil amendments

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Abstract

This article presents a comprehensive on-site bioremediation of an excavated diesel fuel-contaminated soil aided by various soil amendments and plant species. In the first 125 days, the excavated soil was placed in a land treatment unit, mixed with compost, and seeded with white clover (*Trifolium repens*). In the next 155 days, the land treatment unit was re-established and divided into four experimental plots. Experimental plots 2 and 3 were seeded with a mixture of plant seeds (*Trifolium alexandrinum*, *Fagopyrum esculentum* Moench, *Trifolium incarnatum*, *Phacelia tanacetifolia*, *Sinapis alba*, *Trifolium repens*), while experimental plots 1 and 4 were left without seeded vegetation. Moreover, a zeolite and an *Ascophyllum nodosum*-based biostimulant were added to experimental plots 3 and 4. The objectives were to select the most beneficial plant species for the plant-based bioremediation process, to evaluate applicability of different soil amendments for an enhanced hydrocarbon biodegradation, and to study their effects on the microbial community in soil. Our results showed an overall reduction in hydrocarbon pollution by more than 95% within 280 days. The rates of hydrocarbon degradation and changes in the microbial population were not affected by the presence of the zeolite and the biostimulant. In addition, the use of different plant species did not have a statistically significant effect on hydrocarbon degradation but affected microbial population dynamics, confirming stable and diverse indigenous hydrocarbon-degrading microbial community in the native soil.

Keywords Microbial community · Phytoremediation · Soil pollution · Waste management · Zeolite

Introduction

According to the European Commission study in support of the Commission's guidelines on the definition of back-fill, more than 530 million tons of soil was excavated and reported as waste in 2018, two-thirds of which was recycled and returned to the economy (European Commission

and Directorate-General for Environment, 2020). When the contaminated excavated soil is classified as waste, the waste management hierarchy should be applied and recycling should be prioritized over landfilling (European Commission and Directorate-General for Environment, 2021). Since soil provides major ecosystem services by sequestering carbon or neutralizing pollutants, maintaining healthy soil is not only crucial for food production (Iram et al. 2020).

Establishing effective remediation methods for recycling soils is critical. One of the promising options is the process of bioremediation, in which microorganisms or plants detoxify, degrade, and convert hydrocarbons into CO₂, H₂O, and biomass (Hoang et al. 2021). Bioremediation is considered to be an efficient and cost-effective method for degrading hydrocarbon-contaminated soil (Wu et al. 2017). Bioaugmentation (the inoculation of exogenous degrading microorganisms) and biostimulation (the addition of nutrients and soil amendments to circumvent the metabolic limitations of the native community) represent two main types of bioremediation technology (Wu et al. 2016). Since the process of bioremediation is carried out by various microorganisms

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in the soil, degradation is often the result of a community-interacting microbial population, and the success of bioremediation depends on the ability of these microorganisms to adapt to new environmental conditions (Mishra et al. 2001).

One advantage of using plants in the bioremediation of hydrocarbons is that plant characteristics, such as the root system, facilitate rhizoremediation (Abdullah et al. 2020). Different plant species have been shown to stimulate different types of microorganisms (Vieira et al. 2020) that alter the chemical and physical properties of the soil (Vives-Peris et al. 2020). This has been identified as an enrichment effect of the rhizosphere and is reflected in a different bacterial community composition and an increase in the microbial diversity in the overgrown soil (Fan et al. 2017). Since exogenous microorganisms are often unable to compete with indigenous microbial communities and can therefore quickly disappear from the soil (Karamalidis et al. 2010), the use of plants in bioremediation provides an opportunity to create favourable conditions for the increased growth and activity of hydrocarbon-degrading microorganisms (Martin et al. 2014). Several studies have investigated the effect of biostimulation with nitrogen and phosphorus (Nwankwegu et al. 2016; Wu et al. 2016, 2019) or biochar (Dike et al. 2021; Saeed et al. 2021) on hydrocarbon-polluted soils and demonstrated the resulting enhanced degradation of hydrocarbon compounds. Furthermore, in case of heavy metal pollution, phytoremediation studies utilize different fungi with maize (*Zea mays*) and sunflower plants (*Helianthus annuus*) (Iram et al. 2019) and can ultimately result in production of crops used for biodiesel production (Iram et al. 2022). Another class of additives used for remediation are zeolites. These aluminosilicate minerals with ion-exchange properties (Misaelides, 2011) are widely used in agriculture due to their ability to bind nutrients (Cataldo et al. 2021). Zeolites have been shown to increase hydraulic conductivity, increase yields in acidified soils, and improve soil physicochemical properties (Szatanik-Kloc et al. 2021). Since synthetic chemical fertilizers can lead to infertile soils, natural substitutes represent sustainable solution for restoring soil quality (Jaffri et al. 2021).

This work presents the applicability of land farming (the simplest ex situ bioremediation technique (Azubuike et al. 2016)) with plant-assisted bioremediation for the remediation of diesel-contaminated soil at full scale. The contaminated soil originated from an actual diesel fuel spill caused by a broken valve on a pipeline at a fuel storage facility in Rače, Slovenia. After the spill occurred, the soil was excavated and moved to an impermeable asphalt-covered surface within the fuel storage facility and placed in a land treatment

unit (LTU) that allowed the application of soil management techniques.

The main ambition of this study was to make the soil suitable for reuse and to allow its restoration at a polluted site. Therefore, all 90 tons of the excavated soil was used. The first objective of this study was to select the most beneficial plant species for plant-based bioremediation by investigating their effects on the hydrocarbon-degrading microbiological community. The second objective of this study was to evaluate the applicability of different soil amendments, namely compost, zeolite, and a biostimulant, to increase hydrocarbon biodegradation rates. In addition, this study aims to demonstrate how the selected plant species affect the hydrocarbon-degrading microbiological community and enables evaluation of applicability of different soil amendments during the full-scale bioremediation process.

This study started on 26 February 2021 with the LTU establishment and finished on 3 December 2021 with the last soil sampling event. Bioremediation study took place within the fuel storage facility Rače, Slovenia (WGS84 coordinate system; lon.: 15,65963, lat.: 46,45764).

Materials and methods

Study design and the establishment of the land treatment unit (LTU)

After the leak occurred, the soil (67 m³) contaminated with diesel fuel was excavated partially mechanically and partially manually (complete mechanical excavation was not possible due to the underground installations). The excavated soil contained an upper humus layer with grass cover and was classified as Eutric Cambisol on Pleistocene loam (Vrščaj et al. 2017). After excavation, the concentration of hydrocarbon content (C10–C40) was determined to be $8,185.0 \pm 625.0$ mg/kg DW.

In the first part of this study, the excavated soil was placed on an impermeable asphalt surface, mixed with compost (5 m³; JP Voka Snaga d.o.o., Ljubljana, Slovenia), and surrounded with a wooden frame to prevent soil scattering. White clover (*Trifolium repens*) was seeded on the LTU, which also served as an indicator of water stress and as a fertilizer after the soil had been mechanically turned over. As soon as the LTU was established, a manually operated watering regime was introduced (a fire truck equipped with a water tank) to prevent the drying of the soil. The plant-assisted bioremediation with *Trifolium repens* took place for 125 days. After that, the soil was mechanically turned over and the vegetation was mixed into it.



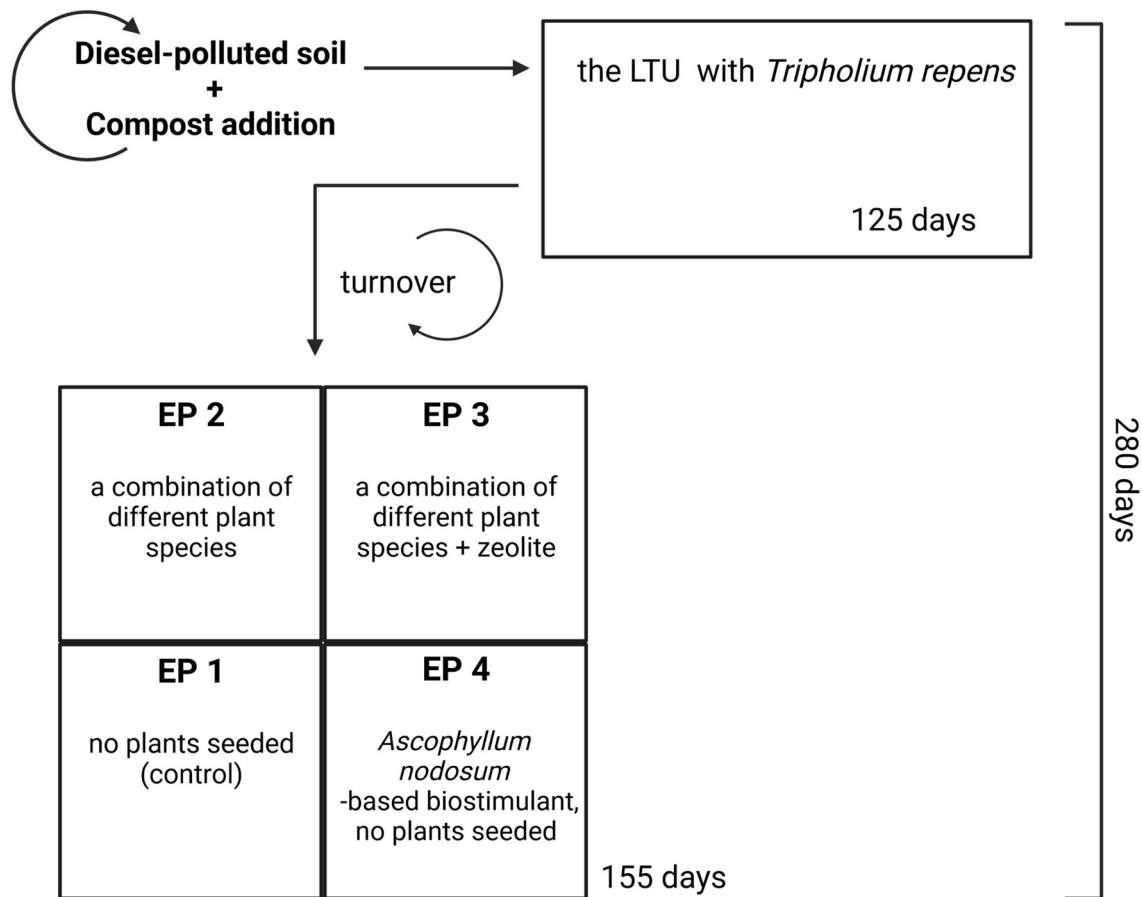


Fig. 1 A graphical presentation of the 280-day bioremediation in LTU. Created with biorender.com

In the second part of this study, which lasted for 155 days, the LTU was re-established and divided into four experimental plots (EPs), each measuring 10 m² (Fig. 1). EP 2 and EP 3 were seeded with the following mixture of plant seeds: *Trifolium alexandrinum* 15%, *Fagopyrum esculentum* Moench 25%, *Trifolium incarnatum* 15%, *Phacelia tanacetifolia* 15%, *Sinapis alba* 15%, and *Trifolium repens* 15% (the mass percentage of seed weight; purchased from Semenarna Ljubljana, Ljubljana, Slovenia).

EP 1 and EP 4 were not seeded. 1,100 g of ZP-4A zeolite obtained from Silkem d.o.o., Kidričevo, Slovenia (the zeolite properties are presented in Tables S1 and S2 in Supplementary Information), was mixed into EP 3, while EP 4 was fertilized with the *Ascophyllum nodosum*-based biostimulant BCX (EKO GEA, Celje, Slovenia).

In a period of one month, plant species from the *Poaceae* family overgrew EP 1 and EP 4. Figure 1 presents a graphical representation of the study design.

The soil was mechanically turned over twice by an excavator to achieve better mixing and homogenization. The first turnover was performed prior to the establishment of the LTU (when the polluted soil was mixed with compost) and the second turnover after 125 days.

During the 280-day bioremediation process, the microbial population was monitored using phospholipid fatty acid (PLFA) analysis. Direct insight into hydrocarbon degradation was obtained by measuring the hydrocarbon content in the soil. Soil nutrient contents were also monitored during the study, namely total nitrogen, total organic carbon, total carbon, and aqua regia-digested phosphorus. Photographs of the LTU at different time intervals during the 280-day bioremediation period are presented in Table S3 in Supplementary Information.



Soil sampling and chemical analysis

Soil samples were collected according to ISO 18400-203, using a soil auger to a depth of 20 cm following a simple systematic pattern. Each composite sample was collected with at least 16 increments. To reduce the edge effect (the difference between biotic and abiotic factors at the edge of a fragmented habitat compared to the interior environment (Ruwanza, 2018), all samples were collected at a distance of 1 m from the edge of the LTU or 1 m from the boundary with the adjacent EP. The efficiency of hydrocarbon degradation was calculated from the measurements of the hydrocarbon content in the soil. The latter was determined according to EN ISO 16703 (Determination of extractable compounds in the range of hydrocarbons C10-C40), using a gas chromatography (GC) method with FID detection.

Phospholipid fatty acid (PLFA) analysis

The chemicals used for the PLFA analysis (acetic acid, acetone, chloroform, dipotassium hydrogen phosphate (K_2HPO_4), n-hexane, potassium hydroxide, methanol, sodium sulphate, and toluene) were of analytical grade and were supplied by Merck KgaA (Darmstadt, Germany). The deionized water used in the experiments was prepared in accordance with SIST EN ISO 3696:1998.

Before the extraction of the PLFAs, 2.5 g of fresh soil samples was sieved through a 2-mm sieve. The PLFAs were extracted using the modified method of Bligh and Dyer with a phosphate buffer, chloroform, and methanol (0.8:1:2 V: V: V, V= volume) (Bligh and Dyer 1959). The lipids were separated through a solid phase extraction column (2 g; 6 mL, Silicycle Quebec City, Quebec, Canada) and transesterified by mild alkaline methanolysis. The remaining fatty acid methyl esters (FAMES) were dissolved in n-hexane and transferred quantitatively.

The FAMES were identified using an Agilent 6890 gas chromatograph (GC, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with an Agilent 5973 mass selective (MS) detector (Agilent Technologies, Inc., Santa Clara, CA, USA). A Supelco 37-component FAME mixture was used for the identification of FAMES.

The sum of all identified PLFAs was used to estimate the total viable microbial biomass (Zhang et al. 2019), and the PLFAs listed in Table 1 were used to determine the presence and abundance of the specific microbial groups (Hedrick et al. 2005; Butterly et al. 2011; Zhu et al. 2012; Willers et al. 2015; Chen et al. 2019). The PLFA nomenclature used in this study was adopted after Zhao et al. (2014). The total number of carbon atoms is on the left side of the colon and the number of double bonds on the right side of the colon, while ω indicates the number of double bonds from the methyl end of the molecule. The *cis* and *trans* configurations

Table 1 The PLFAs determining specific microbial groups extracted from soil samples in this study

Microbial group	PLFA nomenclature
General bacterial marker	14:0; 15:0; 16:0; 17:0; 18:0
Actinomycetes	10Me18:0
Gram-positive bacteria	i13:0; i14:0; a14:0; a16:0; i15:0; a15:0; i16:0 6Me14:0
Gram-negative bacteria	15:1 ω 6c; 18:1 ω 7t; 16:1 ω 7c; 17:1, cy17:0; 16:1 ω 11c
Fungi	18:1 ω 9c; 18:2 ω 6c; 18:3 ω 6c; 18:3 ω 3

Table 2 Soil properties at Day 1 (after the establishment of the LTU)

Parameter	Method	Result	
pH extraction with $CaCl_2$	EN 15933:2012	7.56	± 0.07
Specific electrical conductivity [$\mu S/cm$]	SIST-TS CEN/TS 15937:2013	228.7	± 69.0
Fraction > 2.000 mm [%]	EN 16179:2013	13.3	± 1.3
Fraction < 2.000 mm [%]		86.7	± 8.7
Fraction < 0.002 mm (clay) [%]	ISO 17892—4:2016	1.30	± 0.13
Fraction 0.002–0.050 mm (silt) [%]		62.9	± 6.3
Fraction 0.050–2.000 mm (sand) [%]		28.0	± 2.8
Total carbon [% DW]	EN 15936:2012—method A	4.74	± 0.71
Total organic carbon [% DW]	EN 13137:2002—method B	4.53	± 0.79
Nitrogen [% DW]	EN 16168:2013	0.306	± 0.05
Soil texture*	ISO 17892—4:2016	silt loam	/

*Division between silt and sand at 0.05 mm



are indicated by *c* and *t*, respectively. The *anteiso* and *iso* branching is designated by the prefixes *a* or *i*.

Soil properties

The determined properties of soil one day after the establishment of the LTU and after the addition of compost to the polluted soil are presented in Table 2, indicating the methods used for the chemical analysis.

Data analysis

All results of the PLFA analysis were performed in triplicate and are presented as a mean $\pm$ standard deviation. The principal component analysis (PCA) and all statistical analyses were performed in Microsoft Excel using the XLSTAT package. The PCA was used to evaluate the changes between the microbial communities. Only the PLFAs listed in Table 2 were used for the PCA.

Results and discussion

Soil represents an ecosystem and is therefore modified by the interactions between the individual chemical, biological, and physical components and by their distribution in the soil matrix (Tate, 2020). Therefore, the effect of a single variable

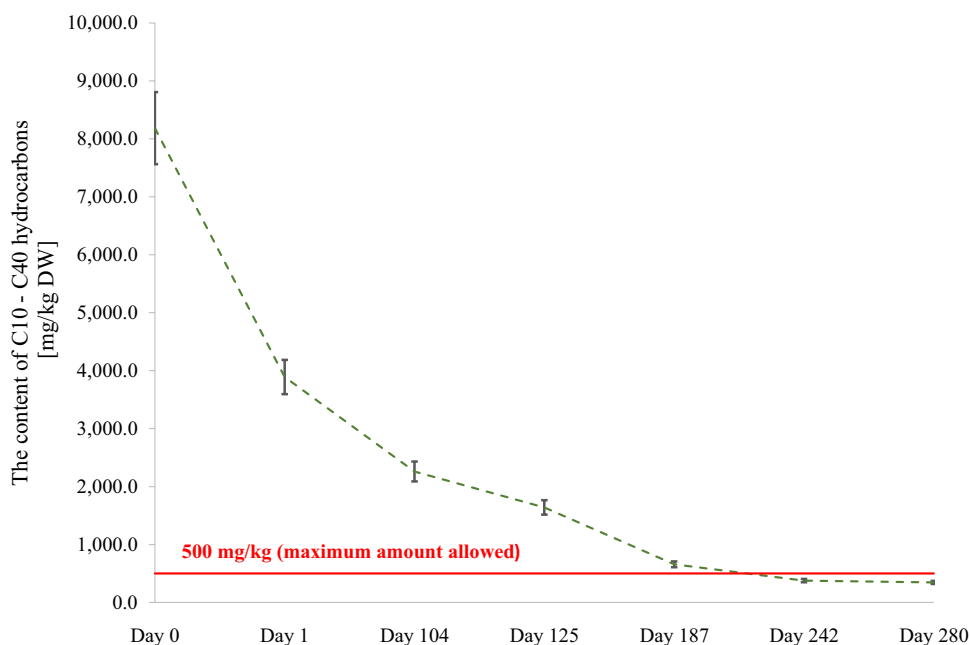
on the process of bioremediation must be considered with caution.

Since this was an on-site bioremediation study, it was inevitably influenced by the natural environment (the climate and weather). At first, an endemic plant species, *Trifolium repens*, was seeded, while in the second part of the study (after 125 days), a mixture of plant species was chosen to increase biodiversity, since this can improve the provision of ecosystem services (Quijas et al. 2010). After 125 days, the LTU was divided into four EPs. Although two of them were not seeded, after approximately one month, both were completely covered with common species of the *Poaceae* family, mainly *Digitaria* sp., *Cynodon dactylon*, *Dactylis glomerata*, and *Echinochloa crus-galli*.

Soil hydrocarbon content during the study

This study shows how a full-scale on-site bioremediation technique can be effective for the hydrocarbon degradation of soil using widely available soil amendments. More than 95% of the hydrocarbon content in soil was removed, reaching a level lower than the legal maximum allowed by Slovenian legislation (Slovenije, 2011). Figure 2 shows how the hydrocarbon content in the soil (C10–C40 mg/kg DW) decreased from the time of pollution until the end of the 280-day study. According to the Slovenian Decree on Burdening Soil by Spreading Waste, the maximum allowable amount of C10–C40 for fillers and soil restoration is 500 mg/kg DW. The study was terminated when the hydrocarbon content

Fig. 2 The values of hydrocarbons C10–C40 during the study (from Day 125, the values represent the average values obtained from all four EPs.)



reached values below this legally permissible level on two separate sampling dates in all four EPs.

A similar large-scale bioremediation was performed in an Arctic landfarm by Johnsen et al. (2021). Their results showed a 64% reduction in diesel content in the first year. However, it must be pointed out that the two climates and soil types are not directly comparable. At Day 280, the hydrocarbon levels had decreased to 346.0 ± 115.3 mg/kg DW (the average value calculated from all four EPs), representing a 95% reduction in diesel contamination. After the completion of the study, the soil in the LTU was re-sampled as excavated soil (waste) according to EN 14899:2006, and a full waste assessment was performed (OC 141-1/2021 by IKEMA d.o.o.). According to that waste assessment, the content of hydrocarbons was determined to be 264.0 ± 79.2 mg/kg DW.

Similarly, as reported by different scientific groups (Namkoong et al. 2002; Osei-Twumasi et al. 2020; Lee et al. 2021), this study confirmed the applicability of compost as a soil amendment for the bioremediation of hydrocarbons. The effect of compost on bioremediation processes is best observed from Day 1 to Day 125, with a 58% reduction in the total hydrocarbon content. Compost is usually added to support the microbial activity of soil microorganisms and to increase their diversity (Gomez and Sartaj 2014). In addition, compost is rich in nutrients such as phosphorus, nitrogen, and carbohydrates, which are believed to be important for the optimal activity of pollutant decomposers (Ferrari et al. 2019).

The significant decrease in the hydrocarbon content on Day 1, after the LTU establishment, can be explained by the dilution of the soil and the addition of compost. In the first 125 days, the hydrocarbon content was reduced by 58.00%,

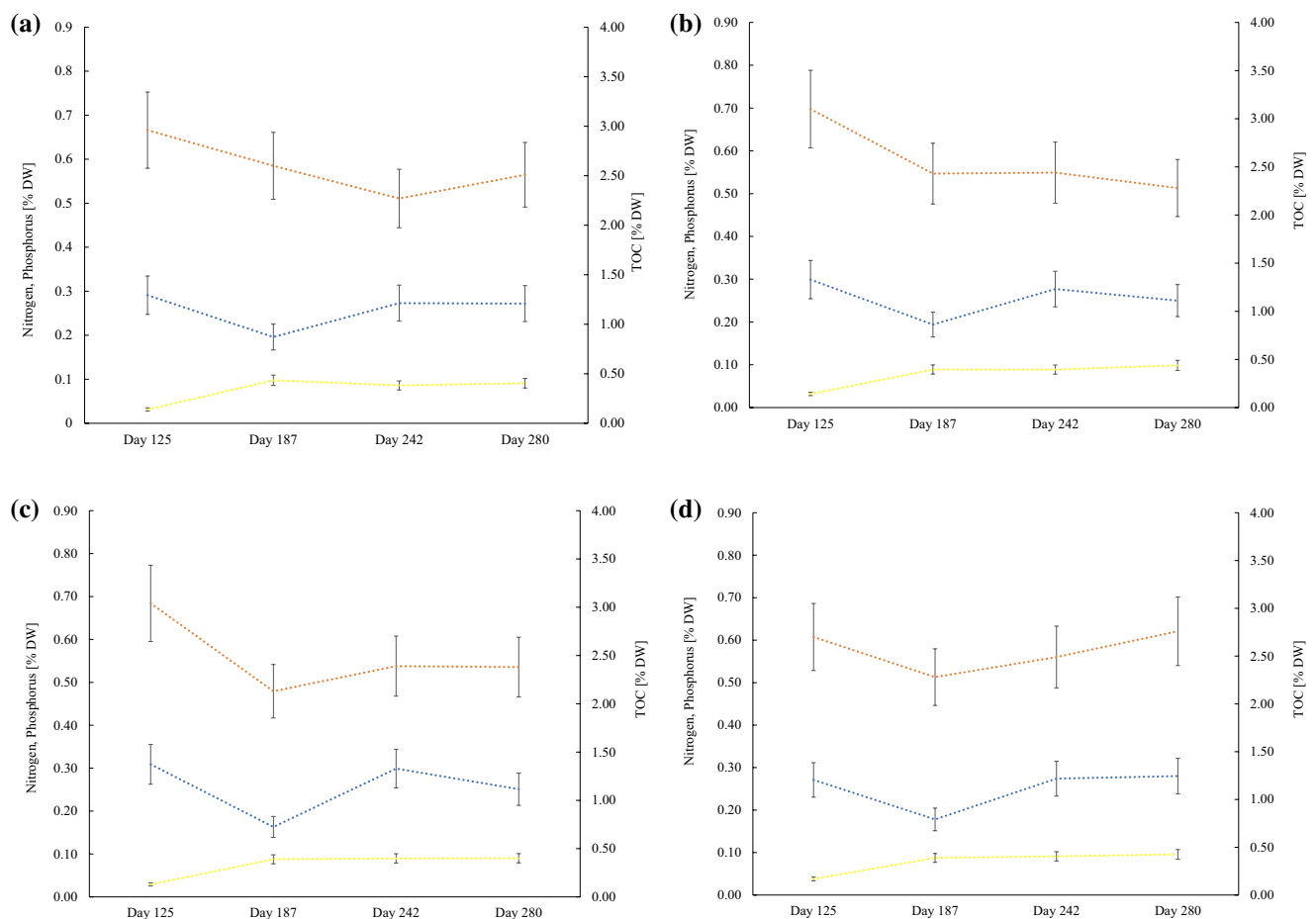


Fig. 3 The TOC (orange), total nitrogen (blue), and aqua regia-digested phosphorus (yellow) changes during the second part of the bioremediation process in EP 1 (a), EP 2 (b), EP 3 (c), and EP 4 (d)



while the efficiency of hydrocarbon reduction from Day 125 to Day 280 was 33.00%. (Since there were no statistically significant differences between the hydrocarbon values for the EPs, the hydrocarbon removal efficiency was calculated as an average value of C10–C40 from all four EPs of the LTU.)

The effect of zeolite, the biostimulant, and different plant species

The importance of nutrients for bioremediation has long been recognized as they support a variety of microbial processes. Nitrogen, potassium, and phosphorus are required for amino acid production and energy transport (Hussain et al. 2018). Microbial population in soil maintains its biomass stoichiometry by partitioning carbon, nitrogen, and

phosphorus between growth and release in the form of CO₂, inorganic nitrogen, and phosphorus (Spohn, 2016).

Data on the total nitrogen, phosphorus digested with aqua regia, and the total organic carbon (TOC) from Day 125 to the end of the study (Day 280) are shown in Fig. 3: EP 1 (a), EP 2 (b), EP 3 (c), and EP 4 (d). A decrease in the TOC was observed throughout the remediation process, while the aqua regia- digested phosphorus increased over time. The changes in the total nitrogen content between the four EPs and between the different time points were not statistically significant ($p < 0.05$).

After the establishment of the LTU, there was a decrease in the TOC content in the soil, while the total nitrogen content was rather constant, ranging between 0.30% DW and 0.20% DW. The rate at which soil organic matter increases and decreases is determined by the gains and losses of carbon in soil (Nair, 2002), but for the successful degradation of

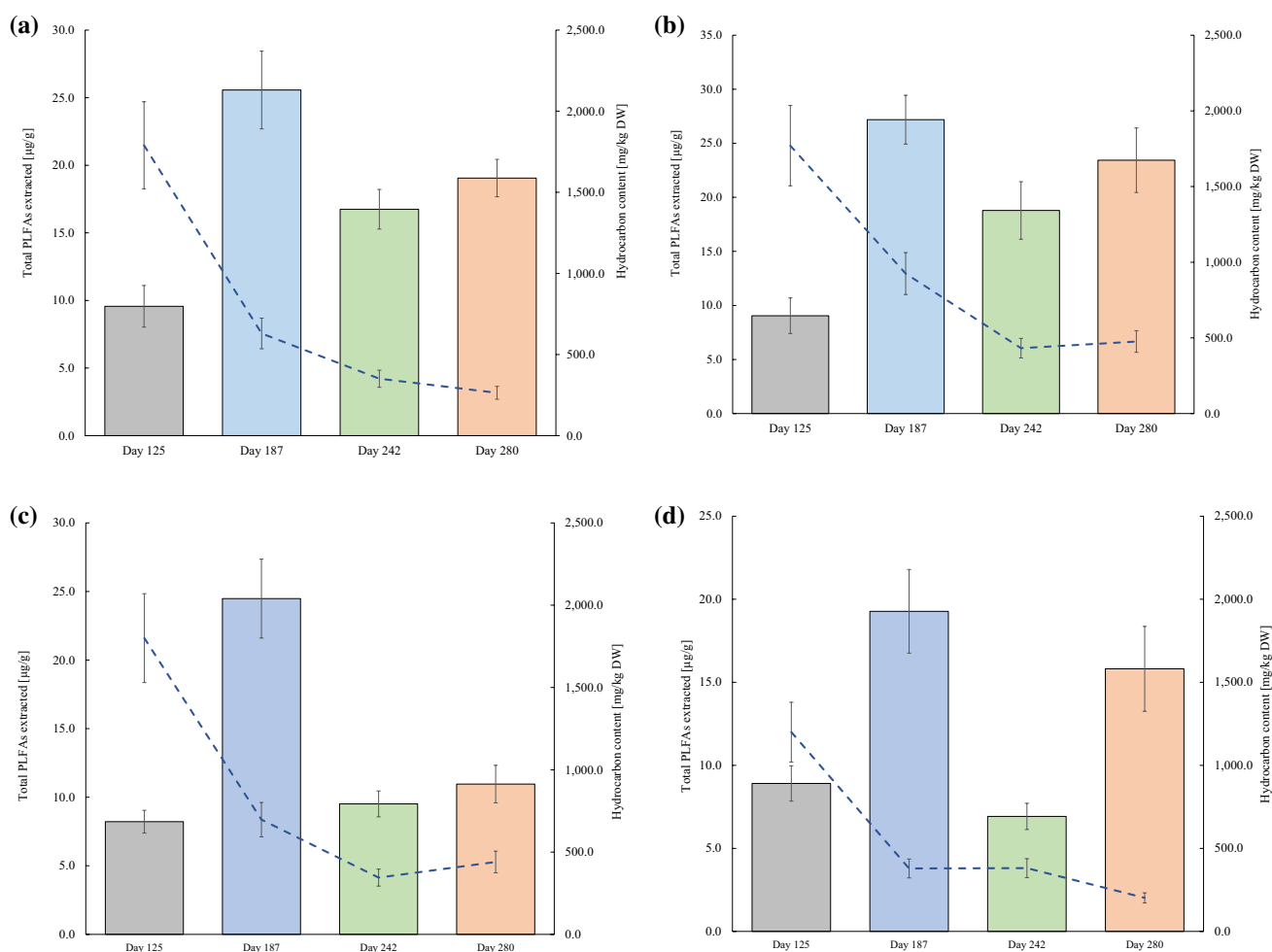


Fig. 4 The hydrocarbon content (dashed line) and amount of total PLFAs extracted during the second part of the bioremediation process in EP 1 (a), EP 2 (b), EP 3 (c), and EP 4 (d)



the target compounds, the added carbon source must remain non-preferential (Namkoong et al. 2002). For the bioremediation of soil, the optimal C/N/P ratio is considered to be 100:10:1 (Banerjee et al. 2016; Wu et al. 2016). The C/N/P ratio was close to this value (100:13:0.8) at Day 125 of this study. After that, the C/N/P ratio changed in favour of phosphorus (100:8:3). The content of phosphorus in soil depends on various factors affecting its availability. Plants uptake phosphorus for growth, but plant species differ in their ability to acquire, use, and retain nutrients. In general, grasses have lower concentrations of tissue phosphorus compared to forbs (Moenclaey et al. 2022), based on which it can be conceded that increased values of this element are a consequence of adding organic matter (i.e. *Trifolium repens*) to the soil when it was mechanically turned over.

The microbial population dynamics during hydrocarbon biodegradation

The use of well-adapted microbes in oil-contaminated soils is considered to be an effective means of bioremediation (Raghavan and Vivekanandan 1999; Tyagi et al. 2011). Since the contamination at issue occurred inside a fuel storage facility due to a valve malfunction and the contaminated soil was naturally present at that site, we assume that the indigenous microbial population was somewhat adapted

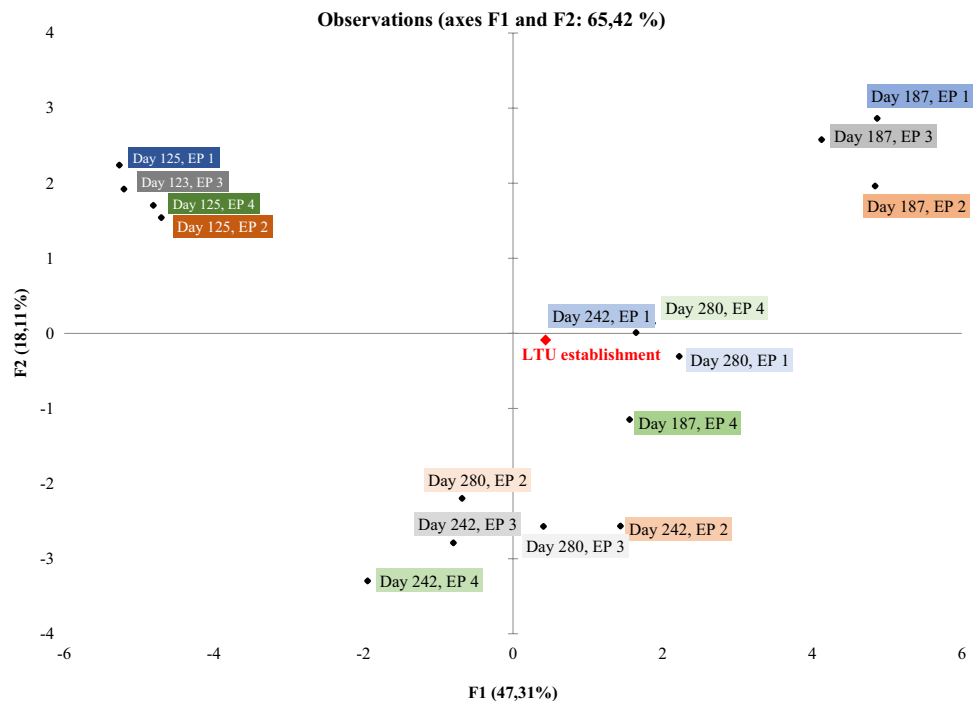
to using hydrocarbons as a source of carbon. For example, Karamelidis et al. conducted a laboratory-scale study with indigenous microorganisms of petroleum-contaminated soil and showed that such an approach results in a > 79% w/w reduction in the total aliphatic hydrocarbons (Karamalidis et al. 2010).

Figure 4 shows the changes in the hydrocarbon content and the amount of total PLFAs extracted from the LTU from Day 125 in EP 1 (a), EP 2 (b), EP 3 (c), and EP 4 (d). A significant increase in total living biomass was observed on Day 187 for all EPs, while the lowest amount of extracted PLFAs in EP 4 was determined on Day 242.

Independent *t* tests for two sample means were performed to reveal potential statistically significant differences ($p < 0.05$; p = significance level) in the hydrocarbon content between pairs of EPs. Such a test was performed for EP 3 (the application of zeolite and the presence of mixed vegetation) and EP 2 (the use of mixed vegetation); EP 4 (the use of the biostimulant) and EP 1 (the control); and EP 1 (no seeded vegetation) and EP 2 (mixed vegetation). ANOVA was also performed to test between-sample variances of the four EPs. None of the tests proved any statistical differences in the sets of data.

To further assess microbial dynamics during the bioremediation process, specific microbial groups were analysed using the PCA. PCA (Fig. 5) revealed that the two

Fig. 5 The PCA showing the microbial population dynamics during the degradation of hydrocarbons in the LTU



principal components, PC1 and PC2, explained 47.31% and 18.11% of the total variance, respectively. Except for Day 1, all subsequent sampling was performed after the LTU had been divided into four EPs, and the influence of zeolite, the biostimulant, and different plant species on the plant-assisted bioremediation was studied (the effects are seen only on Day 187 and later). The PCA plot shows a clustering of the samples collected on Day 125, confirming the low variability among them. The samples taken on Day 125 show the microbial population after the application of compost, in the presence of *Trifolium repens* as supporting vegetation.

Figure 5 also shows several shifts in the microbial population that reveal some dynamics during the bioremediation process. First, the data obtained on Day 187 for EP 1, EP 2, and EP 3 show minimal variance among these samples and a displacement along the F1 axis compared to the data obtained on Day 125, indicating changes in the microbial community during these 62 days. Second, the samples from EP 1 on Days 242 and 280 reveal low between-sample variability compared to the samples from EP 4 on Days 187 and 280, and their common feature is that they were obtained from EPs naturally overgrown by *Poaceae* family species. Third, the samples EP 2 and EP 3, obtained from EPs where a mixture of the selected plant species grew, appear to be in the lower part of the F2 axis. The samples collected on Day 242 from EP 4 show greater similarity to the samples collected on Days 242 and 280 from EP 2 and EP 3. However, in the 38 days between Days 242 and 280, the microbial population in EP 4 became more similar to the microbial population in EP 1 collected on Days 242 and 280.

PCA revealed similarities between microbial populations in plots of soil where mixed plant species were seeded and those obtained from plots where *Poaceae* species grew. Since the PCA for the data obtained on Day 187 did not reveal statistical differences for EP 1, EP 2, and EP 3, but a shift in the microbial population occurred and the highest living biomass was observed for all four EPs, a contribution to the increased living biomass cannot be attributed to soil amendments or plant species. It was hypothesized that this effect was caused by the soil being mechanically turned over, the intensive growth of vegetation in the LTUs, and the addition of *Trifolium repens* to the soil matrix. On Day 280, the variability between the microbial populations for EP 1 and EP 4 was very low, confirming no significant effect of the biostimulant on the microbial population. The similarities between the plots of the LTU with the same type of vegetation confirmed the effect of plant species on the microbial

population, which was also shown in previous studies (Berg and Smalla 2009).

The effect on hydrocarbon degradation during the bioremediation process has already been reported for certain plant species, such as the *Medicago sativa* (Fu et al. 2012; Zhang et al. 2012) and *Poaceae* family species (Hou et al. 2015; Shahzad et al. 2016). On the other hand, the use of combinations of plant species might have various effects on the process of bioremediation. The fibrous root system of grasses (the *Poaceae* family) creates a higher surface area, which improves the contact between pollutants and the degrading microbes (Hussain et al. 2018). In addition, the root system is influenced by the availability of nutrients, which depends on the degree of competition for resources with the neighbouring plants (Rajaniemi, 2022). Furthermore, plants belonging to the *Fabaceae* family can be used to fix atmospheric nitrogen and may have a competitive advantage over other plant species in obtaining additional nitrogen through a symbiotic relationship with *Rhizobium* (Hall et al. 2011). To conclude, according to our data on the hydrocarbon content and PLFA analysis, the choice of plant species affected the dynamics of the microbial population, but not the degradation rates of hydrocarbons in soil, confirming relatively stable and diverse indigenous microbial community of hydrocarbon degraders in soil.

Conclusion

This study demonstrated that >95% of the diesel fuel pollution at issue was removed from the soil within 280 days. The use of compost as a soil amendment provided the nutrients required for the successful degradation of the hydrocarbons by indigenous microorganisms. The seeding of different plant species during plant-assisted bioremediation influenced the microbial population in the soil. However, the choice of plant species did not have a statistically significant effect on the rate of the degradation of the hydrocarbons during bioremediation, confirming relatively stable and diverse indigenous microbial community of hydrocarbon degraders in soil. At the end of this study, a zero-waste policy was achieved and approximately 90 tons of soil was remediated in a fuel depot. It should be noted that landfarming units occupy a large area and can potentially play an important role by adding to urban green spaces, becoming visually appealing elements, or providing a source of food



for pollinating organisms when they are seeded or planted with nectar- and pollen-producing plants.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13762-023-05304-x>.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval Not applicable.

Informed consent Not applicable.

Trial registration Not applicable.

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References

- Abdullah SRS, Al-Baldawi IA, Almansoori AF, Purwanti IF, Al-Sbani NH, Sharuddin SSN (2020) Plant-assisted remediation of hydrocarbons in water and soil: application, mechanisms, challenges and opportunities. *Chemosphere* 247:125932
- Azubuikwe CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques-classification based on site of application: Principles, advantages, limitations and prospects. *World J Microbiol Biotechnol* 32:180
- Banerjee A, Dutta S, Mondal S, Roy A (2016) Bioremediation of hydrocarbon—a review. *Int J Adv Res* 4:1303–1313
- Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol Ecol* 68:1–13
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37:911–917
- Butterly CR, McNeill AM, Baldock JA, Marschner P (2011) Changes in water content of two agricultural soils does not alter labile P and C pools. *Plant Soil* 348:185–201
- Cataldo E, Salvi L, Paoli F, Fucile M, Masciandaro G, Manzi D, Masini CM, Mattii GB (2021) Application of zeolites in agriculture and other potential uses: a review. *Agronomy* 11:1547
- Chen H, Zhao X, Lin Q, Li G, Kong W (2019) Using a combination of PLFA and DNA-based sequencing analyses to detect shifts in the soil microbial community composition after a simulated spring precipitation in a semi-arid grassland in China. *Sci Total Environ* 657:1237–1245
- Dike CC, Shahsavari E, Surapaneni A, Shah K, Ball AS (2021) Can biochar be an effective and reliable biostimulating agent for the remediation of hydrocarbon-contaminated soils? *Environ Int* 154:106553
- European commission & directorate-general for environment (2020) Study to support the preparation of Commission guidelines on the definition of backfilling: Final report, Publications Office
- European commission & directorate-general for environment (2021) Communication from the commission to the european parliament, the council, the European economic and social committee and the committee of the regions EU soil strategy for 2030 reaping the benefits of healthy soils for people, food, nature and climate [Online]. Available: https://ec.europa.eu/environment/publications/eu-soil-strategy-2030_en [Accessed]
- Fan K, Cardona C, Li Y, Shi Y, Xiang X, Shen C, Wang H, Gilbert JA, Chu H (2017) Rhizosphere-associated bacterial network structure and spatial distribution differ significantly from bulk soil in wheat crop fields. *Soil Biol Biochem* 113:275–284
- Ferrari DG, Pratscher J, Aspray TJ (2019) Assessment of the use of compost stability as an indicator of alkane and aromatic hydrocarbon degrader abundance in green waste composting materials and finished composts for soil bioremediation application. *Waste Manage* 95:365–369
- Fu D, Teng Y, Luo Y, Tu C, Li S, Li Z, Christie P (2012) Effects of alfalfa and organic fertilizer on benzo[a]pyrene dissipation in an aged contaminated soil. *Environ Sci Pollut Res Int* 19:1605–1611
- Gomez F, Sartaj M (2014) Optimization of field scale biopiles for bioremediation of petroleum hydrocarbon contaminated soil at low temperature conditions by response surface methodology (RSM). *Int Biodeterior Biodegrad* 89:103–109
- Hall J, Soole K, Bentham R (2011) Hydrocarbon phytoremediation in the family Fabaceae—a review. *Int J Phytorem* 13:317–332
- Hedrick D, Peacock A, White D, Margesin R, Schinner F (2005) Interpretation of fatty acid profiles of soil microorganisms. In: Margesin R, Schinner F (eds) *Monitoring and assessing soil bioremediation*. Springer, Berlin, Heidelberg



- Hoang SA, Lamb D, Seshadri B, Sarkar B, Choppala G, Kirkham MB, Bolan NS (2021) Rhizoremediation as a green technology for the remediation of petroleum hydrocarbon-contaminated soils. *J Hazard Mater* 401:123282
- Hou J, Liu W, Wang B, Wang Q, Luo Y, Franks AE (2015) PGPR enhanced phytoremediation of petroleum contaminated soil and rhizosphere microbial community response. *Chemosphere* 138:592–598
- Hussain I, Puschenreiter M, Gerhard S, Schöftner P, Yousaf S, Wang A, Syed JH, Reichenauer TG (2018) Rhizoremediation of petroleum hydrocarbon-contaminated soils: Improvement opportunities and field applications. *Environ Exp Bot* 147:202–219
- Iram S, Basri R, Ahmad KS, Jaffri SB (2019) Mycological assisted phytoremediation enhancement of bioenergy crops *Zea mays* and *Helianthus annuus* in heavy metal contaminated lithospheric zone. *Soil Sedim Contam Int J* 28:411–430
- Iram S, Iqbal A, Ahmad KS, Jaffri SB (2020) Congruously designed eco-curative integrated farming model designing and employment for sustainable encompassments. *Environ Sci Pollut Res* 27:19543–19560
- Iram S, Tariq I, Ahmad KS, Jaffri SB (2022) *Helianthus annuus* based biodiesel production from seed oil garnered from a phytoremediated terrain. *Int J Ambient Energy* 43:1763–1771
- Jaffri SB, Ahmad KS, Jabeen A (2021) Biofertilizers' functionality in organic agriculture entrenching sustainability and ecological protection. Woodhead Publishing, United Kingdom
- Johnsen AR, Boe US, Henriksen P, Malmquist LMV, Christensen JH (2021) Full-scale bioremediation of diesel-polluted soil in an Arctic landfarm. *Environ Pollut* 280:116946
- Karamalidis AK, Evangelou AC, Karabika E, Koukkou AI, Drinas C, Voudrias EA (2010) Laboratory scale bioremediation of petroleum-contaminated soil by indigenous microorganisms and added *Pseudomonas aeruginosa* strain Spet. *Biores Technol* 101:6545–6552
- Lee YY, Seo Y, Ha M, Lee J, Yang H, Cho KS (2021) Evaluation of rhizoremediation and methane emission in diesel-contaminated soil cultivated with tall fescue (*Festuca arundinacea*). *Environ Res* 194:110606
- Martin BC, George SJ, Price CA, Ryan MH, Tibbett M (2014) The role of root exuded low molecular weight organic anions in facilitating petroleum hydrocarbon degradation: current knowledge and future directions. *Sci Total Environ* 472:642–653
- Misaelides P (2011) Application of natural zeolites in environmental remediation: a short review. *Microporous Mesoporous Mater* 144:15–18
- Mishra S, Jyot J, Kuhad RC, Lal B (2001) Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge-contaminated soil. *Appl Environ Microbiol* 67:1675–1681
- Moeneclaey I, Schelfhout S, Vanhellemont M, Decock E, van Coillie F, Verheyen K, Baeten L (2022) Species ecological strategy and soil phosphorus supply interactively affect plant biomass and phosphorus concentration. *Basic Appl Ecol* 62:1–11
- Nair PKR (2002) The nature and properties of soils 13th edition. In: Brady NC, Weil RR (eds) *Agroforestry systems*. Springer, New York
- Namkoong W, Hwang EY, Park JS, Choi JY (2002) Bioremediation of diesel-contaminated soil with composting. *Environ Pollut* 119:23–31
- Nwankwegu AS, Orji MU, Onwosi CO (2016) Studies on organic and in-organic biostimulants in bioremediation of diesel-contaminated arable soil. *Chemosphere* 162:148–156
- Osei-Twumasi D, Fei-Baffoe B, Anning AK, Danquah KO (2020) Synergistic effects of compost, cow bile and bacterial culture on bioremediation of hydrocarbon-contaminated drill mud waste. *Environ Pollut* 266:115202
- Quijas S, Schmid B, Balvanera P (2010) Plant diversity enhances provision of ecosystem services: a new synthesis. *Basic Appl Ecol* 11:582–593
- Raghavan PUM, Vivekanandan M (1999) Bioremediation of oil-spilled sites through seeding of naturally adapted *Pseudomonas putida*. *Int Biodeterior Biodegrad* 44:29–32
- Rajaniemi TK (2022) Root allocation and foraging precision in heterogeneous soils. *Basic Appl Ecology* 60:25–33
- Ruwanza S (2018) The edge effect on plant diversity and soil properties in abandoned fields targeted for ecological restoration. *Sustainability* 11:140
- Saeed M, Ilyas N, Jayachandran K, Gaffar S, Arshad M, Sheeraz Ahmad M, Bibi F, Jeddi K, Hessini K (2021) Biostimulation potential of biochar for remediating the crude oil contaminated soil and plant growth. *Saudi J Biol Sci* 28:2667–2676
- Shahzad A, Saddiqui S, Bano A (2016) The response of maize (*Zea mays* L.) plant assisted with bacterial consortium and fertilizer under oily sludge. *Int J Phytorem* 18:521–526
- Slovenije VR (2011) Uredba o obremenjevanju tal z vnašanjem odpadkov [Online]. Uradni List Republike Slovenije. Available: <http://www.pisrs.si/Pis.web/pregledPredpisa?id=URED4791> [Accessed]
- Spohn M (2016) Element cycling as driven by stoichiometric homeostasis of soil microorganisms. *Basic Appl Ecol* 17:471–478
- Szatanik-Kloc A, Szerement J, Adamczuk A, Jozefaciuk G (2021) Effect of low zeolite doses on plants and soil physicochemical properties. *Materials (basel)* 14:2617
- Tate RL (2020) *Soil Microbiology*. Wiley, New York
- Tyagi M, da Fonseca MM, de Carvalho CC (2011) Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation* 22:231–241
- Vieira S, Sikorski J, Dietz S, Herz K, Schrupf M, Bruehlheide H, Scheel D, Friedrich MW, Overmann J (2020) Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. *ISME J* 14:463–475
- Vives-Peris V, de Ollas C, Gomez-Cadenas A, Perez-Clemente RM (2020) Root exudates: from plant to rhizosphere and beyond. *Plant Cell Rep* 39:3–17
- Vrščaj B, Repe B, Simončič P (2017) The Soils of Slovenia. *Soil Sci Ann* 71(2):158–164
- Willers C, van Rensburg PJJ, Claassens S (2015) Phospholipid fatty acid profiling of microbial communities—a review of interpretations and recent applications. *J Appl Microbiol* 119:1207–1218
- Wu M, Dick WA, Li W, Wang X, Yang Q, Wang T, Xu L, Zhang M, Chen L (2016) Bioaugmentation and biostimulation of hydrocarbon degradation and the microbial community in a petroleum-contaminated soil. *Int Biodeterior Biodegrad* 107:158–164
- Wu M, Li W, Dick WA, Ye X, Chen K, Kost D, Chen L (2017) Bioremediation of hydrocarbon degradation in a petroleum-contaminated soil and microbial population and activity determination. *Chemosphere* 169:124–130



- Wu M, Wu J, Zhang X, Ye X (2019) Effect of bioaugmentation and biostimulation on hydrocarbon degradation and microbial community composition in petroleum-contaminated loessal soil. *Chemosphere* 237:1244–56
- Zhang J, Lin X, Liu W, Wang Y, Zeng J, Chen H (2012) Effect of organic wastes on the plant-microbe remediation for removal of aged PAHs in soils. *J Environ Sci* 24:1476–1482
- Zhang Y, Zheng N, Wang J, Yao H, Qiu Q, Chapman SJ (2019) High turnover rate of free phospholipids in soil confirms the classic hypothesis of PLFA methodology. *Soil Biol Biochem* 135:323–330
- Zhao C, Xing M, Yang J, Lu Y, Lv B (2014) Microbial community structure and metabolic property of biofilms in vermifiltration for liquid-state sludge stabilization using PLFA profiles. *Biores Technol* 151:340–346
- Zhu YJ, Hu GP, Liu B, Xie HA, Zheng XF, Zhang JF (2012) Using phospholipid fatty acid technique to analysis the rhizosphere specific microbial community of seven hybrid rice cultivars. *J Integr Agric* 11:1817–1827

