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Perennial crop growth in oil-contaminated soil in a boreal climate

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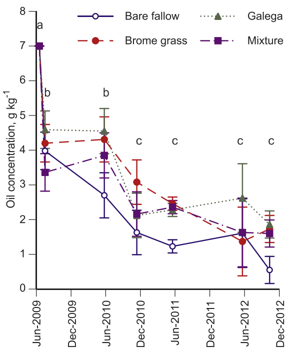
HIGHLIGHTS

- Fodder galega and smooth brome were grown in motor oil contaminated soil.
- 40 months after the oil spike 8%–27% of the oil remained in soil.
- Oil degradation followed first-order kinetics and was fastest in bare fallow.
- Oil increased crop dry matter and nitrogen yield.
- Inoculated fodder galega could fully replace nitrogen fertilizer for brome grass.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil contamination by petroleum hydrocarbons is a global problem. Phytoremediation by plants and their associated microorganisms is a cost-effective strategy to degrade soil contaminants. In boreal regions the cool climate limits the efficiency of phytoremediation. The planting of oil-tolerant perennial crops, especially legumes, in oil-contaminated soil holds promise for great economic benefits for bioenergy and bio-fertilizer production while accelerating the oil degradation process. We established a multi-year field experiment to study the ecological and agronomic feasibility of phytoremediation by a legume (fodder galega) and a grass (smooth brome) in a boreal climate. In 40 months, soil oil content decreased by 73%–92%, depending on the crop type. The oil degradation followed first-order kinetics with the reduction rates decreasing as follows: bare fallow > galega–brome grass mixture > brome grass > galega. Surprisingly, the presence of oil enhanced crop dry matter and nitrogen yield, particularly in the fourth year. The unfertilized galega–brome grass mixture out-yielded the N-fertilized pure grass swards over years by an average of 33%. Thus, a perennial legume–grass mixture is both ecologically and agronomically sustainable as a cropping system to alleviate soil contamination in the boreal zone, with considerable potential for bioenergy and bio-fertilizer production.

1. Introduction

Soil pollution by petroleum hydrocarbons (PHCs) is an increasing problem around the world. In Finland, for example, the number of contaminated sites grew from 10,400 in 1994 to 23,850 in 2013 (Pyy et al., 2013). In situ bioremediation using indigenous microbes is an effective and low-cost strategy to degrade contaminants, but this process is limited by microbial activities, the biochemistry of enzymes, the

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resistant nature of the contaminants, and site-specific factors (Balba et al., 1998; Boopathy, 2000). Plants are able to enhance the bioremediation of oil-polluted soil by stimulating soil microflora (Radwan et al., 1995; Suominen et al., 2000; Acharya et al., 2015). The efficiency of this phytoremediation relies on the establishment of healthy plants with sufficient shoot and root biomass growth to support the activities of a flourishing microbial consortium at the rhizosphere (Wenzel, 2009). Dense cultivation of suitable crops in polluted sites was thus suggested as a promising approach for bioremediation (Radwan et al., 1995).

Nutrient deficiency, however, particularly that of nitrogen and phosphorus, often limits biodegradation in contaminated sites (Wenzel, 2009). Legumes, due to their capacity for symbiotic biological nitrogen fixation, can do without N fertilizer input, thus assisting in the bioremediation of soils contaminated with petrochemical waste (Kamath et al., 2004; Chiapusio et al., 2007). Since bioremediation is a slow process that does not allow many disturbances of the contaminated soil, the use of perennial legumes with proper field management holds promise for accelerated oil degradation. Fodder galega (*Galega orientalis* Lam.), a fast-growing perennial forage legume, and smooth brome (*Bromus inermis* L.), a cool-season perennial sod-forming grass, are both persistent in boreal and nemoral zones and have been shown to grow well together in crop mixtures (Jasinskas et al., 2008; Kryževičienė et al., 2008). The oil tolerance and rhizoremediation potential of *G. orientalis* and its microsymbiont *Neorhizobium galegae* to remediate oil-contaminated soils have been demonstrated at microcosm and mesocosm scales (Suominen et al., 2000; Lindstrom et al., 2003; Jussila et al., 2006; Mikkonen et al., 2011).

For these reasons, we set up a systematic, field-scale study on bioremediation of oil-contaminated soil coupled with plant biomass production in a boreal region. We established a multi-year field experiment to investigate the ecological suitability and potential economic benefits of fodder galega and smooth brome to grow in and bioremediate an oil-contaminated soil, and to develop an integrated and sustainable system for long-term cost-effective bioremediation practice in boreal and nemoral climates.

2. Materials and methods

2.1. Experimental design and climatic conditions

The field experiment was established at the Viikki Experimental Farm, University of Helsinki, Finland (60°14′N, 25°01′E, 8 m AMSL) (Table 1). It was a split-plot experimental design in four replicate blocks, with four crop treatments (pure brome grass, pure galega, galega–brome grass mixture, and bare fallow) as the main plot factor (Fig. S1). The sub-plot factor was factorial combinations of oil spiked (7000 ppm) and unspiked treatments with plant growth promoting bacteria (PGPB) inoculated and un-inoculated treatments, providing 64 plots in all (4 crop treatments × 2 oil treatments × 2 PGPB treatments × 4 replicates). The mean temperatures of the growing seasons (May–October) exceeded the long-term (1971–2000) average by 2.1 °C in 2010 and 1.8 °C in 2011, and the precipitation exceeded the average in 2009, 2011 and 2012 (Table 2).

Table 1
General information about the experimental field.

Site properties	Details
Site area	420 m^2
Plot size	3.75 m^2 (2.5 m × 1.5 m)
Soil structure	Clay loam (on average, 32% clay, 36% silt, 32% sand)
Altitude	8 m
Vegetation zone	Boreal
Annual precipitation	650 mm
Annual mean temperature	4.9 °C
Farming systems	Integrated
Cropping history	*Salix*

2.2. Treatment preparation and field management

2.2.1. Field management

The site was treated with two herbicides: glyphosate (N-(phosphonomethyl)glycine), a broad-spectrum systemic herbicide, before the establishment of the plots in June 2009 and Basagran® SG (165 g/50 l) for post emergence broad-leaved weed control in September 2009. Weeds growing in the experimental plots were removed manually in the growing season, except for a second glyphosate treatment in June 2011 in the bare fallow plots, although this treatment had no visible effect. Buffers between the blocks were maintained as weedy grassland to prevent edge effects and other disturbances between neighboring plots. Mineral N fertilizer (60 kg ha^{-1} of N as urea) was given to the pure grass plots in the summer of 2009. In May 2012, mineral N fertilizer was given only to the PGPB-treated pure grass plots. The legume plots and legume–grass mixtures received no N fertilizer throughout the experiment.

2.2.2. Oil spike

The spiking experiment was performed to evaluate the biological toxicity of the oil hydrocarbons and to assess the overall bioremediation efficiency. The oil was a mixture of used motor engine oil (Teboil Lubricants Classic Mineral Motor oil, SAE 10 W-30, API SF/CD, Finland), with a density of 0.877 kg l^{-1} at 20 °C, according to the manufacturer. The target contamination was 7000 ppm (7 g kg^{-1}) of motor oil in soil, assuming a soil bulk density of 1.0 g ml^{-1}. For each oil-spiked plot, 6 kg of oil was mixed with 10 kg of white coarse sand (0.5–1.2 mm), spread, and mixed into the top 20 cm of soil in the oil-treated plots with a rotary tiller on 17 June 2009. 10 kg of pure sand without oil was mixed into the top 20 cm of soil in the control plots.

2.2.3. Seed co-inoculation and sowing

Before sowing, commercial seeds of *G. orientalis* cv. 'Gale' (Naturcom Oy, Ruukki, Finland) and *B. inermis* cv. 'Lehis' (Jõgeva Plant Breeding Institute, Estonia) were surface-sterilized before inoculation with bacteria. To ensure biological nitrogen fixation, all galega seeds were inoculated with *N. galegae* strain HAMBI 540 (University of Helsinki, Helsinki, Finland). Two plant growth promoting root-colonizing bacteria strains, *Pseudomonas trivialis* 3Re27 (Graz University of Technology, Graz, Austria) and *Pseudomonas extremorientalis* TSAU20 (National University of Uzbekistan, Uzbekistan), were inoculated onto the seeds of both crops as described by Egamberdieva et al. (2010). The PGPB-free seeds were used as controls. The inoculated seeds were mixed with peat prior to sowing. The seeds were manually sown and lightly covered by raking. The first sowing was done on 7 July 2009. Brome grass was sown at 35 kg ha^{-1}, galega at 25 kg ha^{-1}, and the combination at 26 kg ha^{-1} of brome grass and 6 kg ha^{-1} of galega to give a 75:25 ratio. Due to a poor initial growth of the galega, it was resown in May 2010.

2.3. Crop biological measurements and data handling

The crops were cut with a forage harvester twice in a growing season over three successive growing seasons (2010–2012). The first cut was done when flowering began in late June and the second cut was done in late August, these being typical harvesting times for hay or silage. The total fresh biomass of crops (W) was weighed on the day of harvesting and the species in the mixtures were separated. The proportion of galega (G%) was estimated on the basis of fresh weight in each mixture plot. Crop dry matter content (DM%) was determined by drying to the constant mass at 105 °C. The DM yield (t ha^{-1}) of each harvest was calculated as follows: DM yield = DM% × W. The total DM yield in the