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The effect of electrokinetics on soil microbial communities

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Abstract

There is growing interest in the potential of applying an electric field to soil to move and stimulate the degradation of contaminants, but we know little of the impact of this approach on exposed microbial communities. The effect of electrokinetics (3.14 A m^{-2}) on soil bacterial and fungal communities was studied using soil cartridge microcosms (13 cm × 5.4 cm × 5.9 cm). After 27 days of electrokinetics, a zone of low pH (<4) was detected close to the anode. Soil exposed to electrokinetics and immediately adjacent to the anode demonstrated an increase in carbon substrate utilisation potential (≤290%) and microbial respiration rates. The diversity and structure of the bacterial community showed little response to electrokinetics, with the exception of soil close to the anode. Here, an increase in the percentage of Gram-positive species isolated was identified, most notably of *Bacillus megaterium*. Overall, the only detectable response of the microbial community was observed in soil immediately adjacent to the anode. The results of this study provide evidence that the application of electrokinetics has no serious negative effect on 'soil microbial health', thus endorsing its validity as a viable soil remediation technology.

Keywords: Electrokinetic; Soil remediation; Soil health; Microbial community; Diversity; Activity

1. Introduction

A key requirement for any new methods for the bioremediation of contaminated soil is to demonstrate its effectiveness in reducing the level of contamination present. However, if the remedial procedure proves to be effective in reducing the pollution load, but to the detriment of exposed indigenous microbial communities, then the procedure is unlikely to receive universal acceptance. An important requirement before new methods for the bioremediation of contaminated land are accepted is to demonstrate that they are effective for clean-up but, as importantly, that they do not have any negative effects on microbial communities and therefore soil health.

One of the most recently introduced procedures for remediating contaminated land is soil electrokinetics, which has successfully been used to stimulate the degradation of soil contaminants (Maini et al., 2000) and facilitate the removal of heavy metals (Kim et al., 2002). The underlying mechanism by which the process is effective is the introduction of a direct current into soil. This causes hydrogen ions to be generated at the anode and hydroxyl ions at the cathode, so a pH gradient develops between the electrodes. Furthermore, the introduced current leads to the migration of contaminant, by several processes including electromigration, electrophoresis and electroosmosis (Lageman et al., 1989). It is the processes of electromigration and electrophoresis that together results in the movement of ions and ion complexes and charged particles, respectively, towards the electrode of the opposite charge to the contaminant. Furthermore, electroosmosis results in the movement of soil moisture or, in some applications, groundwater towards the cathode in response to an electric charge. As transportation occurs, the extraction and removal of contaminants may be achieved by precipitation or ion exchange at the electrode, or by pumping out contaminated water close to the electrodes, resulting in decontamination. Thus, electrokinetically induced contaminant migration has been demonstrated to be successful, both in and ex situ, exciting interest in the commercial opportunities of its

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application to a wide range of pollutants, both metals and organics (Maini et al., 2000).

Because of the growing commercial interest in electrokinetics, it is increasingly important to determine the impact of this process on exposed soil microbial communities and, potentially on soil health. As described above, electrokinetics inevitably impacts on soil conditions (Acar and Alshawabkeh, 1993); indeed, this is the very basis by which the process works. Parameters, such as pH and soil water content, which are critical to soil microbial community composition and processes, certainly are impacted, as may soil temperature, which can increase by 1–3 °C as a result of electrokinetics. Furthermore, electric fields of sufficient strength can have a direct effect on microbial cells. In this case, the effect on microbial cells may be due to damage to the cell membrane. To our knowledge, there have been no reported studies to determine the effect of direct current (DC) electrokinetics on the composition, community structure and functional diversity of exposed soil microbial communities.

The aim of this study was to employ a combination of complementary approaches, including phenotypic (culture with chemotaxonomic analysis) and genotypic methods, to determine the composition and structure of a soil microbial community exposed to continuous DC electrokinetics for 27 days within a laboratory soil cartridge microcosm.

2. Materials and methods

2.1. Soil electrokinetic cells

Soil was taken from the Oxford University field station at Wytham, Oxford, from a depth of between 20 and 50 cm. The soil is an Evesham Series heavy clay with 53% clay, 25% sand and 22% silt (Lilley et al., 1996a) and with an organic content of ~8.5%. All samples were air-dried and sieved (<2 mm) prior to use to facilitate the even packing of the electrokinetic chambers and improve the sample homogeneity. The soil was remoistened to a water content of 18–21% by adding small amounts of de-ionised water. Soil was stored in the dark at 4 °C for 1 month, allowing the soil biology and physicochemistry to recover from the drying process.

Six microcosms were constructed (similar to Harbottle et al., 2001), consisting of acrylic cartridges (Fig. 1) containing 0.5 kg of wet soil that was statically compacted at a vertical stress of 50 kPa. Distilled, deionized water (400 ml) was added uniformly to each cartridge and the leachate collected and recycled three times. The soil was then left for 24 h to dry to a saturation ratio of 70%. A direct electrical current (3.14 A m^{-2}) was applied to three of the electrokinetic chambers using Isotech IPS601A benchtop power supplies (Southport, UK) connected to graphite electrodes (Fig. 1) submerged in water. Three additional chambers were similarly constructed, but without the application of an electrical current. Treatments are referred to in the text as 'Electrokinetic' and 'Control', denoting chambers with or without the applied current, respectively. During experimentation, fluid lost from the chambers by electroosmosis was continually replaced to the anolyte, to maintain a constant fluid level across the electrode chambers.

2.2. Soil sampling

Soil cores (~3 g wet weight) were removed for microbial analysis from the electrokinetic chambers 0, 9, 18 and 27 days after the current was applied, from the positions shown in Fig. 1. On day 0, samples were removed before the application of the electric current. During the study,

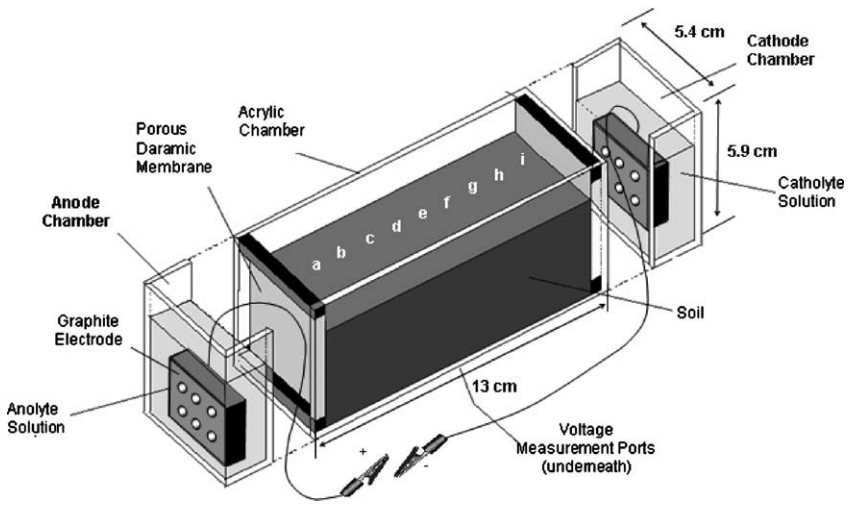

Fig. 1. Schematic of the sampling regime within the electrokinetic chamber. Nine samples were taken across the length of each chamber (a, nearest to anode; i, nearest to cathode; $a = 0.72$ cm from anode, $b = 2.17$ cm from anode, etc, in increments of 1.44 cm) 0, 9, 18 and 27 days after the current was applied. Chamber dimensions: 13 cm × 5.4 cm × 5.9 cm. Graphite electrode dimensions: 5.0 × 5.0 × 0.8 cm.

electrolyte pH values were routinely monitored using pH indicator paper (BDH Ltd, UK), and sulphuric acid (1 M) was added to the cathode chamber to maintain a catholyte pH of around 7. This had the effect of maintaining electro-osmotic flow throughout the experiment. Soil temperature was measured using a pH/temperature microcomputer (HI 9025; Hanna Instruments Ltd, UK). Soil water content was routinely determined across the sample chambers for each sampling point by drying soil at 105 °C to a constant dry weight. Soil conductivity and pH were determined at the final sampling time. For each sample, 10 g (wet weight) of soil was combined with 60 ml of distilled water, and glass beads (borosilicate, 5 mm; Sigma-Aldrich Company Ltd, UK) and vortexed (3000 rpm, 1 min); soil pH was measured, using a pH probe, whilst the slurry was continually stirred. Soil conductivity was monitored using a Conductivity meter (HI8733; Hanna Instruments Ltd, UK) applied to the still slurry once particulates had settled. Pore water conductivity (saturation extract) was monitored by placing 50 g of air-dry soil into a beaker with enough distilled water to form a saturated paste (as described by Rhoades, 1982). After 2 h, a vacuum was applied to draw the saturation extract through a Buchner funnel (Royal Worcester, UK), lined with Whatman No. 50. filter paper (Raymond and Lamb Ltd, UK). To prevent the precipitation of $CaCO_3$, 0.1% sodium hexametaphosphate was added before measuring conductivity. Conductivity was related to salinity by interpolation using a standard curve created with known amounts of NaCl.

2.3. Soil microbial counts

For each soil sample, 1 g was combined with 9 ml of phosphate buffer solution (K_2HPO_4, 0.65 g l^{-1}; KH_2PO_4, 0.19 g l^{-1}), a 10-fold serial dilution prepared, and 30 μl spread onto two media, selected to study bacteria and fungi, respectively, incubated in the dark at 28 °C. Potato dextrose agar (PDA) (Oxoid, CM463) containing 320 mg l^{-1} Aureomycin® (chlortetracycline hydrochloride; Cyanamid, Gosport, Hampshire, UK), added as an antibacterial agent, was used to determine counts of colony-forming units (CFU) of fungi within the soil. Similarly, diluted soil suspensions were plated onto plate count agar (PCA) containing 100 mg l^{-1} cyclohexamide (Sigma-Aldrich Company Ltd, Dorset, UK) as an antifungal agent. Colony counts (after 24 and 48 h, respectively) of bacteria and fungi were expressed per gram of dry soil.

2.4. Soil microbial functional diversity

To determine the response of the microbial community to electrokinetics in terms of functional diversity, soil samples were analysed by incubating diluted soil in BIOLOG EcoPlates™ (Hayward, California, USA). The technique enables the rapid multiple assay of the carbon assimilation potential by microbial communities, allowing both spatial and temporal differences in microbial communities to be determined.

From each soil chamber, three soil samples were removed from sampling positions a, e and i (shown in Fig. 1) at each sampling time. Soil (0.5 g wet weight) was placed into a 15 ml centrifuge tube (Bibby Sterilin Ltd, Staffs) and 10 ml of phosphate buffer saline (PBS; NaH_2PO_4, 2 mM; Na_2HPO_4, 8 mM; NaCl, 130 mM; pH 7.2) was added together with 5 mm glass beads to a total volume of 12.5 ml. This mixture was agitated for 1 min using a bench-top vortex and centrifuged (4000 rpm, 10 min). One millilitre of the supernatant was then combined with 14 ml PBS and again vortexed and centrifuged. The supernatant was discarded and soil was resuspended in 14 ml PBS, and again centrifuged. The pellet was resuspended in 19 ml PBS, and 100 μl of the solution was then added to each well of a BIOLOG EcoPlate™ containing 31 different carbon sources (and a control well with no carbon source). This procedure was previously optimised to provide 10^8 bacterial cells ml^{-1} (as proposed by Haack et al., 1995; Konopka et al., 1998). Plates were incubated at 28 °C and was measured as absorbance of each well at 620 nm using an optical density microplate luminometer (Anthos Lucy 1 (Software v1.5), Labtech International Ltd, East Sussex, UK) every 24 h for 10 days. Absorbency readings from each well were transformed to account for variable rates of colour development, caused by differing inoculation densities (Garland and Mills, 1991), as follows

$$(S - C)/B \times 10^9;$$

where S is the sample well $OD_{620\ nm}$, C is the control well $OD_{620\ nm}$ and B is bacterial CFU g^{-1} oven dry soil.

2.5. Total genetic diversity of soil bacteria

The impact of electrokinetics on the diversity of the bacterial community, including the unculturable component was determined by denaturing gradient gel electrophoresis (DGGE). Soil nucleic acids were extracted using the method of Griffiths et al. (2000) before being resuspended in 200 μl of Tris–EDTA buffer (10 mM Tris–HCl (pH 7.4), 1 mM ETDA (pH 8.0)). The variable region of 16S rDNA was amplified by PCR using universal bacterial primers (GC338F^{orward} 5′-CGC CCG CCG CGC CCC CGC CCC GGC CCG CCG CCC CCG CCC ACT CCT ACG GGA GGC AGC-3′ and 530R^{everse} 5′-GTA TTA CCG CGG CTG CTG-3′) for conserved regions of the genes as detailed by van der Gast et al. (2001). The PCR was performed using a Peltier Thermo Cycler (PTC-225; MJ Research Inc., Watertown, Mass. (USA)) as follows: (i) 95 °C for 5 min; (ii) 35 cycles of 95 °C for 1 min, 60 °C for 30 s, and then 72 °C for 30 s; (iii) 72 °C for 30 min. The presence of amplification products was verified by electrophoresis in 1% agarose gels, stained with ethidium bromide (1×10^{-5}%).

DGGE was performed according to the method of van der Gast et al. (2001), where PCR samples (20 μl) were loaded onto a 10% (w/v) polyacrylamide gel with a 30–60% denaturing gradient, in 0.5% × Tris acetate EDTA (TAE) buffer (pH 8.3, Severn Biotech Ltd, Worcs, UK). Electrophoresis was achieved using the Ingeny Phor-U DGGE system (Ingeny International BV., Goes, NL) for 16 h, at a controlled temperature of 60 °C, and a constant voltage of 100 V (BioRad Powerpac 3000; Biorad, UK). The gels were subsequently stained (for 20 min) in 0.5 × TAE buffer containing 2 mg ml^{-1} Syber Gold (Molecular Probes, Inc., Oregon, USA) and photographed using a GeneGenius (Syngene, Cambridge) gel documentation system.

2.6. Chemotaxonomic analysis

The diversity of cultured soil bacteria exposed to electrokinetics was determined by fatty acid methyl ester (FAME) analysis of the samples taken after 0 and 27 days. FAME analysis allows the characterisation of individual bacterial and fungal colonies. Fatty acids are extracted and compared against a database, to identify isolates to the species level. From each culture plate containing between 30 and 300 colonies, 10 individual colonies were randomly isolated and incubated on tryptic soy broth agar (TSBA, Becton Dickinson & Company, MD, USA) for 48 h at 28 °C. These isolates were identified using the FAME analysis procedure as described by Thompson et al. (1993). Cells (50 mg wet weight) were transferred into sterile, sealed Pyrex™ boiling tubes, saponified with NaOH (1 M) in 50% methanol, and then methylated with 6 M HCl and methanol (11:9). FAMEs were extracted from the acidic aqueous phase and mixed with hexane and methyl-tert-butyl ether (1:1) before the organic phase was separated and the base washed by adding a 0.3 M solution of NaOH. Samples were analysed using a Hewlett-Packard 6890 chromatograph, processed using the Microbial Identification System, MIS (MIDI Inc., Newark, USA), and identified using the MIS 'Aerobe Library' software (Sherlock v3.10, MIDI, Inc., USA).

A number of approaches were used to analyse the soil bacterial diversity and community structure as determined by FAME analysis, includes determining indices of species richness and Simpson's diversity index (Simpson, 1949) (data not shown), and the Shannon-Weiner Index (Renyi, 1987),

$$\text{Shannon-Wiener index}(H') = \sum_{i=1}^{s} (p_i)(\log_e p_i)$$

(s = numbers of species in sample, p_i = proportion of species i in the sample).

2.7. Soil microbial respiration

As a measure of microbial activity, the respiration of soil microorganisms was monitored using a modified version of the method of Öhlinger (1996). Four soil samples were removed from sampling locations b, d, g, and h in the reactor chamber (Fig. 1), for each time-point, and 2 g placed into individual wide screw-neck jars (100 ml, Fisher Scientific UK Ltd, Loughborough). Sterile water was added to each container to adjust the soil samples to 50% water holding capacity. Bijou bottles (Fisher Scientific UK Ltd, Loughborough) containing 5 ml of NaOH (0.05 M) were placed into each pot and vacuum grease was smeared around the thread lids to ensure that the vessels were airtight. Control readings were also included, with four containers established as above, but lacking soil. All samples were incubated at room temperature for 18 h.

After incubation, the NaOH from each bijou bottle was combined with 2 ml of $BaCl_2$ (0.5 M) and five drops of phenolphthalein solution (0.1% in 60% ethanol), before being titrated with 0.1 M HCl until the solution had changed from pink to cloudy white. The volume of CO_2 produced per gram of oven dry soil was then calculated as follows

$$\text{mg } CO_2\ g^{-1} \text{ oven dry soil} = \frac{(c - s) \times 2.2}{\text{soil dry wt (g)}}$$

where c is the volume (ml) of HCl used for the titration of the controls, s is the volume (ml) of HCl used for the titration of the soil samples and 2.2 is a conversion factor.

2.8. Statistical analysis

Bacterial and fungal counts were calculated per gram of oven dry soil and log-transformed ($\log_{10} + 2$) to improve the homogeneity of the variance. Analyses of variance (ANOVA) were performed on the data using the GENSTAT statistical package (GENSTAT V release 6.1). Unless otherwise stated, ANOVAs were constructed using a general treatment structure, with randomised blocks. Phoretix™ 1D Advanced software (Non-linear USA Inc., Durham, NC) was used to construct dendrograms of the DGGE profiles with simple cluster analysis of band presence or absence, utilising the unweighted pair-group method using mathematical averages.

3. Results

3.1. Soil properties

Although slightly higher towards the anode end of the electrokinetic cells, soil temperature generally differed by less than 1 °C along the sampling transect. The pH of the soil close to the anode (2 cm), was significantly ($P < 0.001$) lower than in the control (pH 3.5 and 8.0, respectively; Fig. 2). No significant spatial trend ($P = 0.430$) in soil conductivity was observed across the length of the cartridges, although within the electrokinetic chambers, a 177% increase in total electrical conductivity was recorded throughout the entire cell (Fig. 3) as compared with the control.

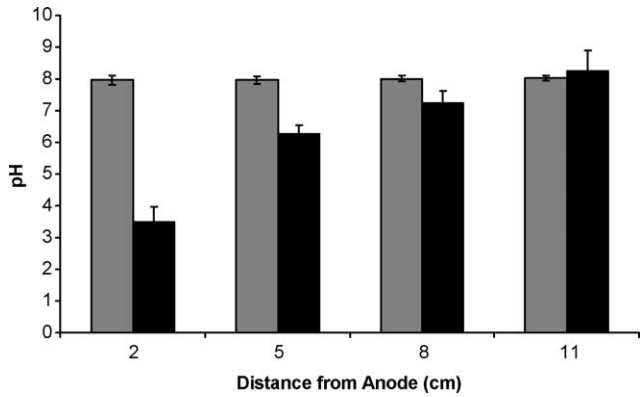

Fig. 2. pH across chamber transect from anode to cathode during final sampling time (27 days). Data are standard pH units; ▒, control; ■, electrokinetic; the results of an ANOVA were 0.960 and <0.001, for each treatment, respectively. Error bars are 1 × SE.

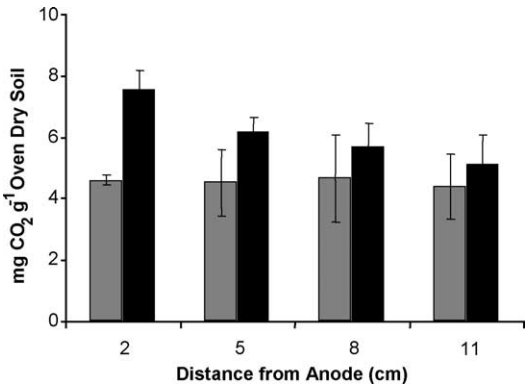

Fig. 4. Effect of treatment (▒, control; ■, electrokinetic) on soil microbial respiration, sampled along the cartridge transect from anode to cathode after 9 days of applied treatment. Data are mg CO_2 respired g^{-1} oven dry soil; the results of an ANOVA are 0.904 and <0.001, for each treatment, respectively. Error bars are 1 × SE.

3.2. Viable counts and soil microbial respiration

Culturable numbers of fungi and bacteria generally remained stable within the control treatment over the period of study (i.e. 6.5 ± 0.03 ($\log_{10}+2$) bacterial CFU g^{-1} oven dry soil, 4.9 ± 0.06 ($\log_{10}+2$) fungal CFU g^{-1} oven dry soil). Fungal counts in control soils showed no significant difference ($P > 0.05$) to those exposed to electrokinetics. Bacterial counts were also not significantly different ($P > 0.05$), except 0.7 cm from the anode and cathode, where counts decreased by a maximum of 18%.

3.3. Soil microbial respiration

Soil microbial respiration was significantly higher ($P < 0.001$) towards the anode end of the electrokinetic chambers after 9 days of application (Fig. 4). However, no significant difference (i.e. $P > 0.05$) was detected at any other sampling time. A significant positive correlation was observed between numbers of bacterial CFU in the soil and microbial respiration ($n = 179$, $R^2 = 0.2278$, $P = 0.01$; all samples combined), although no trend was determined directly between soil pH and microbial respiration.

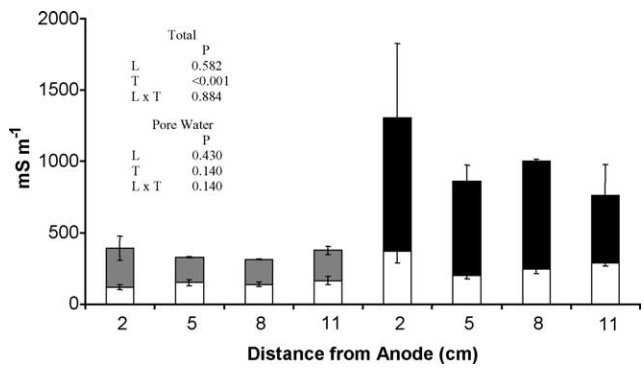

Fig. 3. Soil conductivity across chamber transect from cathode to anode during the final sampling time (27 days). Data are EC_e (mS m^{-1}) determined for the pore water (saturation extract) □ and total soil conductivity; ▒, control; ■, electrokinetic; the results of an ANOVA is shown on the graph (L, sampling position; T, Treatment; *P*, probability). Error bars are 1 × SE.

3.4. Functional diversity of the soil community

The impact of electrokinetics on the functional diversity of the soil microbial community was investigated by examining carbon assimilation patterns as determined by BIOLOG. This revealed the order of carbon substrate utilisation for the soil community was: polymers > amino acids > carbohydrates > amines > carboxylic acids > phenols (as determined by the greatest absorbance (OD_{620}) after 10 days of incubation). However, in soil exposed to electrokinetics, phenols were utilised to a greater extent than amines. Generally, substrate utilisation patterns remained similar within the electrokinetic cells, except those close to the anode (Fig. 5) where the utilisation of amino acids, for example, was 290% greater than in the control cell. Towards the anode (at sampling position (a)), colour development was both more intense and rapid; here, amino acids, carbohydrates and carboxylic acids were preferentially utilised and to a far greater extent than the polymers.

3.5. Genotypic community profiling

Examination of the banding patterns obtained from the DGGE of soil 16S rDNA revealed that samples clustered mainly according to the duration of exposure to electrokinetics, indicating a change in the bacterial community structure over time (Figs. 6 and 7). Analysis of the data by cluster analysis revealed less variation in diversity amongst the control samples than within the electrokinetic chambers (≈0.6 and 0.4, respectively). A divergence was detectable within the electrokinetic cells whereby a greater difference in community composition was noted between sampling

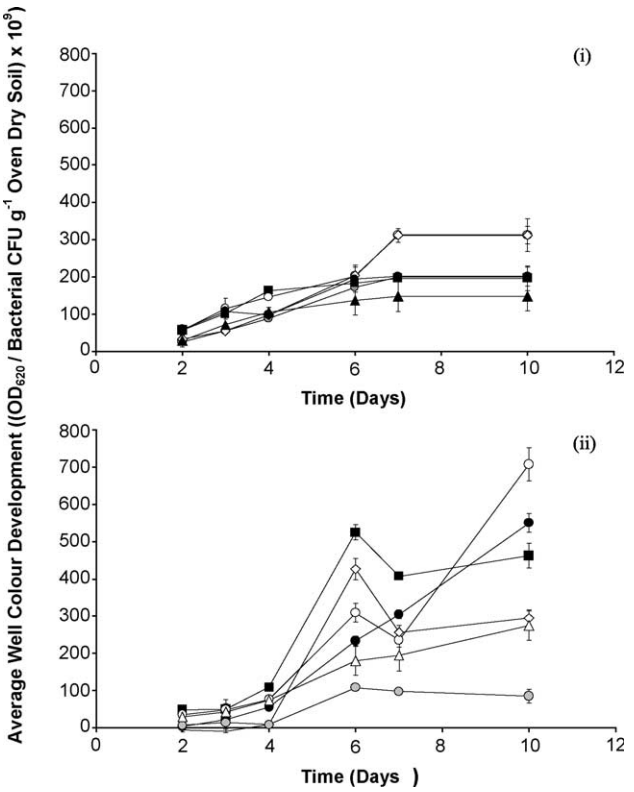

Fig. 5. Colour development profiles (OD_{620}) incubated over a 10-day incubation period for soil taken from sampling location a (0.72 cm from the anode) during the final sampling time (after 27 days). (i) Without electrokinetics (ii) with electrokinetics. Data are transformed OD_{620} values grouped as an average for each of the six carbon substrate types in the BIOLOG EcoPlate: ◇, polymers; ○, amino acids; ●, carbohydrates; ■, carboxylic acids; △, phenols; ◍, amines from the control and electrokinetic treatments (i and ii, respectively). Error bars are 1 × SE.

locations as time progressed, highlighting the spatial changes developing within the soil microbial community over time. However, the most conspicuous finding occurred at the anode end of the chamber, where after 1 week of applied current, there was insufficient PCR product recovered from the soil to provide any detectable band using DGGE.

3.6. Community composition by chemotaxonomy

Comparison of the bacterial community composition of soil by FAME revealed some significant differences (Table 1) after 27 days. Most notably, the percentage of isolates identified to be from the *Bacillus* genus were significantly lower, closer to the anode than the cathode of the electrokinetic cells ($P < 0.05$). In contrast, the highest percentage of *Arthrobacter* detected occurred close to the anode, whilst being undetected in control soils. Significant differences between treatments were detected within the *Bacillus* genus. *Bacillus mycoides* and *Bacillus sphaericus* dominated the bacilli community within the control soil (representing 35 and 22% of the total, respectively), whilst no cells of *B. sphaericus* were identified within the electrokinetic cells. Conversely, the electrokinetic cell contained a higher percentage of *B. megaterium*, which was not detected in the control. The proportion of bacteria isolated that were Gram-negative also decreased between the first and final sampling dates, more so within the electrokinetic cells than in the controls (a 25 and 16% decrease, respectively; data not shown).

Constructing ranked plots of bacterial abundance (data not shown) enables inferences to be made about the structure of the soil microbial communities as suggested by Lilley et al. (1996b). No striking changes in community hierarchy were detected as a consequence of the electrokinetic treatment. Furthermore, although bacterial diversity was most variable within the electrokinetic cells, no clear trend could be observed in terms of diversity over the length of the cartridges, using any of the indices applied.

4. Discussion

To our knowledge, this is the first in-depth study aimed at determining the impact of soil electrokinetics on indigenous soil microbial communities. Although the procedure is applied specifically to move contaminants and stimulate rates of degradation, it is known to alter soil parameters, such as pH, which is critical to microbial growth; it is, therefore, important to determine any unintentional impact on the soil microbial community. Previous investigators have shown that the application of an electric field of 30 kV cm^{-2} to bacteria in a liquid medium can induce rapid mortality of the cells (Ulmer et al., 2002). In contrast, with exception to some notable changes in the activity and composition in the microbial community close to the anode, the applied current caused little detectable impact in this study. However, these two studies differed in two important ways: the intensity of current (which in this study was significantly less (3.14 A m^{-2})) and the presence of the buffering capacity of the soil. Indeed, application of 20 mA cm^{-2} to soils has been shown to stimulate the activity of sulphur-oxidising bacteria (Jackman et al., 1999), which was attributed to the pH changes induced by electrokinetics. Similarly, in this study there is strong evidence to suggest that the significant impact on the soil microbial community and activity that was detected exclusively adjacent to the anode was attributable to the low pH generated by the electrokinetic process.

Soil pH is a crucial parameter for soil microbial growth, influencing a complex range of interacting factors, such as membrane integrity and function, and also the bioavailability of nutrients and contaminants. Typically, the application of electrokinetics induces a pH gradient across the bioreactor cell (Jackman et al., 2001; Mattson et al., 2002). This is due to electrolysis reactions that produce protons at the anode, leading to an acid front that advances towards the cathode. Although the electrolytic generation of hydroxyl ions can be neutralised within the catholyte,

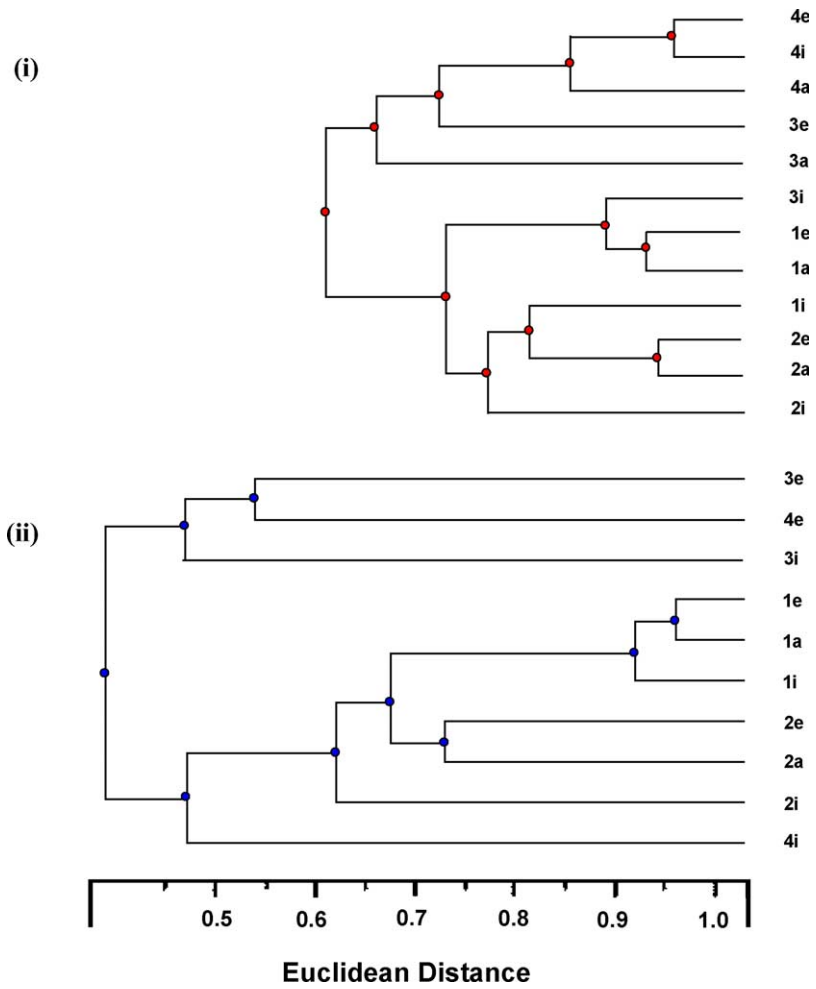

Fig. 6. Genetic similarity of microbial-community profiles obtained with PCR–DGGE, sampled along the cartridge transect from anode to cathode (a, e and i, respectively) for each sampling time (1 → 4; 0, 9, 18 and 27 days, respectively) for both the control (i) and electrokinetic (ii) treatments. Data are Euclidean distance determined from the presence/absence of bands.

a decrease in pH may, as in this study, occur within the soil close to the anode. Differences in both soil temperature and conductivity have also been detected once an electrical current is applied, but it is assumed that the fluctuation in soil pH is the prime cause of changes in soil microbial activity and distribution.

Blagodatskaya and Anderson (1998) determined that lowering soil pH resulted in the reduction of soil microbial biomass, coupled with an associated increase in the specific respiration rate of CO_2, which has been suggested to be due to shifts in microbial community composition (Fleiβach and Mäder, 1997). A number of studies have described changes amongst bacterial communities within acidified soil, which have been shown to alter such factors as the size and activity of the soil microbial biomass (Tabatabai, 1985). As in this study, Thompson et al. (1987) observed an increase in the ability of isolated bacteria to metabolise a range of different substrates within acidified soils. Importantly, it has been demonstrated that changes in soil pH affect soil processes including litter decomposition and carbon mineralisation

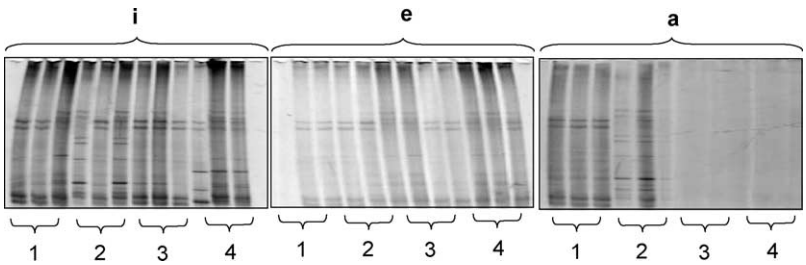

Fig. 7. DGGE gels showing similarity between replicates for each sampling time (1 → 4; 0, 9, 18 and 27 days, respectively) and location (a, e, i) within the electrokinetic cells.

Table 1
Composition of bacterial communities isolated from soil exposed to electrokinetics for 27 days, determined by FAME analysis

Genera	Treatment									
	Electrokinetic					Control				
	0.72	3.60	6.48	9.36	12.24	0.72	3.60	6.48	9.36	12.24
Arthrobacter	1	13		2	2					
Bacillus	2	1	8	15	19	10	11	22	18	8
B. megaterium	1	1		7	11					
B. mycoides			1	4	2	2	3	7	9	3
B. sphaericus						2	7	4	0	2
Microbacterium		4	2	1	4					
Paenobacillus	4		1	2	2	4	2		1	2
Pseudomonas	1	1				5	1	1		2
Variovorax	4		2			1				1
No match		2	2			4	4	2		4
Other[a]	9	3	11	7	5	3	6	2	10	11
Total	21	24	26	27	30	27	24	27	29	28

[a] Includes: *Agrobacterium*, *Alcaligenes*, *Brevibacillus*, *Brevibacterium*, *Brevundomonas*, *Cellulomonas*, *Chromobacterium*, *Comomonas*, *Corynebacterium*, *Deinococcus*, *Enterobacter*, *Exiguobacterium*, *Flavobacterium*, *Kocuria*, *Micrococcus*, *Norcardia*, *Phenylobacterium*, *Staphylococcus*, *Stenotrophomonas*, *Xanthomonas*.

(Kalburtji et al., 1997). Persson et al. (1989) suggested that such responses may be a direct effect of pH or a result of pH altering carbon bioavailability and, thus, selecting for a more active microbial community (Bååth et al., 1995). No increase in bacterial numbers have occurred overall in this study. It is also possible that the observed increase in carbon substrate utilisation potential and respiration rates could have been induced by stress responses to the electric current or the rapidly reduced pH.

Bacteria are known to alter their cell wall or membrane composition in response to environmental changes such as pH, which in turn may alter patterns of gene regulation (Lundström and Bölin, 2000) or induce sporulation as their environment becomes more acidic or stressful. The observed increase in substrate utilisation detected by BIOLOG and microbial respiration may have been a consequence of the need for components of the soil microbial community to maintain their intracellular pH values. Thus, the observed increase in the utilisation of the simpler compounds (i.e. amino acids and carbohydrates) detected for the total microbial community at the anode end of the electrokinetic cell may be a reflection of its requirement for an immediate source of energy to maintain its active transport system. Although temporal patterns in culturable fungal numbers were noted, it is suggested that bacteria contributed most to the observed changes in carbon substrate utilisation and respiration. Most fungi grow too slowly to develop a significant colour response within the BIOLOG plates. Furthermore, bacteria as a group often have higher pH optima compared with fungi (Bååth, 1996) and would, thus, be less adapted to the prevailing conditions within the electrokinetic cell close to the anode.

As well as having the potential to alter the ratio of abundance of bacteria to fungi, relatively rapid declines in pH, as occurred immediately adjacent to the anode, are known to alter the species composition of bacteria (Bååth et al., 1992, 1995). The most significant influence of the application of electrokinetics, detected by FAME, was an increase in the proportion of *B. megaterium* within the soil. This may be explained by the ability of *B. megaterium* to withstand the applied stresses, synthesising a protective capsule containing both polypeptide and polysaccharide to form resting cells after the end of an exponential period of cell growth. Thus, it is possible that conditions within the electrokinetic chamber induced a greater production of endospores, which increased the number of *Bacillus* spp. isolated. The ability of the genus *Arthrobacter* to tolerate environmental stresses (such as desiccation and starvation) is also well known (Gray, 1976), with viable cells frequently recovered from extreme environments and historic sediments (Vorobyova et al., 1997). Increased numbers of *Arthrobacter* isolated from the anode end of the electrokinetic cells may, therefore, have been a consequence of their better survival within the soil close to the anode. However, with the exception of *Arthrobacter* and *Bacillus*, few significant changes in the composition or community structure of the bacterial community were detected; a view further supported by the consistent nature, with exception of the anode, of the DGGE profiles.

Stress responses are likely to be the cause of several of the significant changes within the soil microbial community detected, particularly adjacent to the anode. For instance, increased rates of respiration and metabolic ability, as revealed by BIOLOG, have been observed in soils exposed to pollution stress (Thompson et al., 1999). The greater assimilation ability (BIOLOG) of the soil microbial community at the anode may have been due to the lower pH increasing nutrient availability in the soil, thereby predisposing the extracted cells to utilise a greater range of nutrients in the BIOLOG plates. Changes in soil

conductivity within the electrokinetic chambers may have also accounted for some of the detected community changes. Indeed, both *B. megaterium* and *B. sphaericus* are known to be halotolerant, which may account for the increased percentage of *Bacillus* spp. isolated. However, although the electrokinetic treatment induced a greater soil conductivity, this was thought to be of lesser significance than the changing in soil pH, as the spatial changes amongst the bacterial community from anode to cathode did not correlate with the increased conductivity of the soil throughout the entire cell.

Finally, the effect of the prevailing conditions on the methodology used must not be overlooked. For example, surface-active particles in soil and sediment are known to absorb and bind both organic and inorganic materials, including DNA (Stotzky, 2000). Moreover, at a lower pH, the proportion of soil bound DNA increases, thus increasing the difficulty of extraction. Consequently, the reason for the lack of PCR product obtained from soil taken from the anode end of the electrokinetic chambers is hard to ascertain. This may be due to lower total numbers of bacteria in the soil, providing less genetic material for the PCR reaction, or the soil surface charge could have influenced the extraction process, thereby biasing the community profile. Large fluctuations in pH or ion concentrations may have also thwarted the amplification of the extracted soil nucleic acids.

This experiment forms part of a wider study of the effects of electrokinetics on pollutant bioavailability. Whilst the effect of air-drying and rewetting on the microbial community must not be overlooked (Mondini et al., 2002), the repacked soil system was designed to reduce sample variation inherent when studying a soil taken directly from the field. However, the inclusion of controls and provision of a recovery period is likely to have reduced any of the community changes induced by the soil treatment. Furthermore, study of such an ex situ laboratory-based system facilitated better control and replication, enabling more inferences to be made about the effects of the electrokinetic treatment. Indeed, before progressing to field-based studies, it may prove useful to examine further the dual effect of electrokinetics on the soil microbial community when applied to a contaminated soil ex situ. The current study was undertaken using one soil type, and one set of experimental conditions. However, further studies should examine the effect of electrokinetics on a range of soil types, and at varying soil depth. Of particular interest is the use of electrokinetics below the water table, where groundwater may contribute to the dilution of acid/base within the soil, thereby reducing any impact of electrokinetics on the soil microbial community.

In conclusion, the results of this study demonstrate that the application of electrokinetics to soil alters both the physicochemical characteristics of the soil and the exposed microbial community, with soil acidification near the anode seemingly being the most prominent response. The direct effect of the applied current on soil bacteria could not be ascertained, this influence being inseparable from other electrochemically induced soil changes. We identified a possible bacterial stress response, induced by electrokinetics. However, the observed effect was only detected within soil closest to the anode, with little effect elsewhere in the soil. The effect of many physical soil remediation techniques on soil microbial communities have been poorly studied and, in most cases, ignored. However, provided that factors such as soil pH and temperature are suitably controlled, there is no reason to suggest that the application of electrokinetic technologies should be restricted due to concerns for maintaining soil health. Nevertheless, a better understanding of interactions between soil engineering processes and biology may be a key issue in understanding, and perhaps improving, the efficacy of soil remediation technologies. The maintenance of soil health is needed to enable the continued intrinsic degradation of pollutants by the indigenous microbial populations or bacterial inocula following subsequent pollution events.

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