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Vertical advection of extracellular DNA by water capillarity in soil columns

Maria Teresa Ceccherini[a], Judith Ascher[a], Giacomo Pietramellara[a,*], Timothy M. Vogel[b], Paolo Nannipieri[a]

[a]*Dipartimento Scienza del Suolo e Nutrizione della Pianta, Università degli Studi di Firenze, P.le delle Cascine, 28-50144 Firenze, Italy*
[b]*Ecologie Microbienne UMR CNRS 5557, Université Claude Bernard, Lyon 1, Bat. Mendel, 43, bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France*

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Abstract

The fate of extracellular DNA in the environment concerns both the fate of transgenes from genetically modified organisms and the evolution of active bacteria capable of incorporating this DNA into their genomes. This study addressed the possibility that DNA, like other organic molecules, could move vertically in the capillary fringe of groundwater aquifers. The targeted gene fragment used here was the 35S-*nptII* sequence, which was below detection levels in controls. Initial microcosm studies detected the DNA target molecule by PCR during the entire experiment. The vertical advection of water and DNA were monitored for a period of 3 days in soil columns. DNA was added as a water solution at the bottom of the unsaturated soil column, and then DNA-free water was added at the bottom after 12 and 24 h. After the addition of the DNA solution, capillary water rose 4 cm within the soil column and the target DNA was detected up to that height. After 60 min, the entire soil column (10 cm) was wetted and the target sequence was detected up to a height of 7.5 cm. After the second wetting (12 h later), the target sequence was detected up to the top of the soil column (10 cm). However, after the third wetting (24 h later), the marker sequence was only found at heights from 0.5 to 4 cm. Results clearly show the vertical movement of DNA due the capillary rise and suggest the possibility of DNA degradation within the soil column.

Keywords: Water-capillary rise; Extracellular DNA; Nucleic acid movement; Soil microcosm; PCR monitoring

1. Introduction

Water fluxes in subsurface ecosystems are critical for soil and water management (Droogers et al., 2000; Marinov et al., 2005). These fluxes include groundwater flow, aquifer recharge and capillary rise. In addition to water fluxes, water quality plays an important role in ecosystem quality. While considerable research has dealt with the fate of contaminated water, little has been done concerning water-soluble molecules with important biological functions such as nucleic acids. Minor, but measurable, fractions of dissolved DNA have been shown to move through soil columns if they escape complete degradation by nucleases (Potè et al., 2003; Agnelli et al., 2004). The leaching of dissolved extracellular DNA has been suggested to occur throughout the soil profile. Groundwater recharge (downward infiltration from the unsaturated zone to the aquifer), as well as upward capillary rise (by capillary forces at water equilibrium) (Marinov et al., 2005; Depountis et al., 2001), could move extracellular DNA between soil layers. However, no studies have been carried to verify this hypothesis.

Molecular techniques based on PCR can detect, and therefore, monitor movement of specific extracellular DNA through the soil profile if the DNA target sequence is not naturally present in the soil. Many common marker genes used to monitor DNA are those conferring antibiotic resistance such as *bla TEMI* (ampicilin) (Duggan et al., 2000), *aadA* (spectinomycin–streptomycin) (Ceccherini et al., 2003), *nptII* (kanamycin) (de Vries et al., 2003), (Gebhard and Smalla, 1999), *hp* (igromycin), *cat* (clor-amfenicol) and *aac* (gentamycin) (Bertolla et al., 2000).

*Corresponding author. Tel.: +39 055 3288230; fax: +39 055 333273.
E-mail addresses: mariateresa.ceccherini@unifi.it (M.T. Ceccherini), giacomo.pietramellara@unifi.it (G. Pietramellara).

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While many of these genes are found naturally in soil, their concentrations are often quite low.

The aim of this study was to monitor the possible vertical advection of extracellular nucleic acid in soil by water capillarity. The 35S-*nptII* target sequence (*CaM* promoter and gene conferring resistance to kanamycin) was added to soil columns and the DNA vertical advection by capillarity was monitored under laboratory conditions.

2. Materials and methods

2.1. Soil and DNA

Soil was sampled from the 0–15 cm soil layer at Romola (Italy) in May 2004 and sieved at 2 mm. The soil from this site had the following characteristics: pH 7.2; sand 81.9%; silt 6.7%; clay 11.4%; organic carbon 0.7% and organic nitrogen 0.07% (Badalucco et al., 1997).

The genomic DNA, isolated from *Acinetobacter calcoaceticus*, supplied by Kaare Nielsen, carried the 35S *CaM* promoter and *nptII* gene, conferring the resistance to kanamycin.

Selective LB medium (10 ml) containing kanamycin (50 μg/ml final concentration) was inoculated with one single colony of *A. calcoaceticus* in sterile 50 ml tubes and incubated overnight on a horizontal shaker at 29 °C. Cells were collected as pellets by centrifugation (4 °C, 10 min, 20,800*g*; Centrifuge 5804R, Eppendorf), resuspended in an appropriate volume of sterile distilled water and processed for DNA extraction by the FastDNA™ Kit and instrument (BIO101, Inc.). Yields of extracted DNA were evaluated by fluorometer (DyNA-Quant™200, Hoefer) using bisbenzimide-dye Hoechst H 33258 (Agnelli et al., 2004; Renella et al., 2004) and calf thymus DNA was used as the calibration standard; the size was controlled on agarose gel by electrophoresis.

2.2. Analysis of extracellular DNA in soil

Aliquots of 0.5 g soil were put in 2 ml microcentrifuge tubes, spiked with 2 μg genomic DNA (200 μl in volume) from *A. calcoaceticus* and incubated at room temperature, with the moisture at the water-holding capacity (Badalucco et al., 1997). Controls consisted of three soil tubes inoculated with 200 μl of sterile water and three tubes with dry soil only. After 20 and 60 min, and 1, 3, 10 and 20 days of incubation, extracellular DNA was recovered from soil. Each sampling time was replicated three times. Aliquots of DNA were then analyzed by gel electrophoresis (50 μl) to monitor changes in DNA size, by fluorometer (2 μl) for the DNA quantification and by PCR (10 μl) for the presence of the marker gene.

2.3. Soil column experiments

Soil columns (Fig. 1) consisted of 10 cm high polypropylene tubes with a diameter of 1.5 cm. They were filled with 19 g of air-dried and sieved soil to a height of 10 cm and put in glass containers (3 cm in height, 2.5 cm in diameter). The soil columns were then inoculated with DNA containing the target sequence, as follows. A sterile DNA aqueous solution was prepared to wet 12 soil columns. Three milliliters of the DNA solution, the amount sufficient to wet the whole soil column by capillary rise, was then added to the glass container at the bottom of each soil column. The amount of extracellular DNA added to each soil column was 15 μg DNA for 19 g soil based on calculations after pilot experiments (inoculation of soil with decreasing amounts of extracellular DNA from *A. calcoaceticus*), which indicated that 700 ng g^{-1} soil is the minimum concentration of DNA needed to detect the 35S-*nptII* target sequence by PCR after DNA extraction from 0.5 g of soil. Soil columns were incubated at room temperature. Soil samples were taken at 20 and 60 min ($t_{20'}$ and $t_{60'}$), 1 and 3 days (t_{1d} and t_{3d}) after DNA inoculation. After 20 min, the visible wet part of the soil column was 4 cm high, while after 60 min the entire soil column was wet. After 12 h, 3 ml of sterile water was added to each column in order to continue the capillary rise of DNA. The sample t_{1d} was taken after 16 h. After 24 h, columns were wetted again with 3 ml sterile water. Two days of incubation were necessary for the complete absorption of water by the soil column and thus, the sample (t_{3d}) was collected after 36 h of incubation.

Soil slides of 0.5 cm were cut from the soil column at heights of 0.5, 1, 1.5, 2, 4, 7.5, 8 and 10 cm with a knife as shown in Fig. 1; soil (0.5 g) was immediately analyzed for the content of extracellular DNA and the residual soil sample was stored at −20 °C. Each sampling was replicated three times (three different soil columns) at each incubation time (20 min, 60 min, 1 day and 3 days). Since eight slides at different heights were sampled per column, the overall number of soil samples analyzed was 96. Moreover, control soil columns (three) were also prepared and they were wetted with sterile water and only one soil sample per replicate was analyzed for its DNA content.

2.4. Recovering of extracellular DNA from soil

Extracellular DNA was recovered and purified as described previously (Agnelli et al., 2004): soil (0.5 g) was rinsed with 500 μl of alkaline buffer (0.12 M Na_2HPO_4, pH 8) (Ogram et al., 1987) in 2 ml microcentrifuge tubes for 30 min under gentle shaking at room temperature. The soil suspension was centrifuged at 20,800*g* at 4 °C for 30 min. These steps were repeated three times. The supernatant was collected in a new microcentrifuge tube and mixed with a protein precipitating solution (BIO101, Inc.). Precipitated proteins were eliminated by centrifugation (20 °C, 5 min, 20,800*g*), then the purification of the extracellular DNA extract was performed with a GENECLEAN procedure (BIO101, Inc.) consisting of a three-step bind–wash–elute procedure: DNA was bound on a silica resin, collected by centrifugation in spin columns (20 °C, 2 min, 20,800*g*;

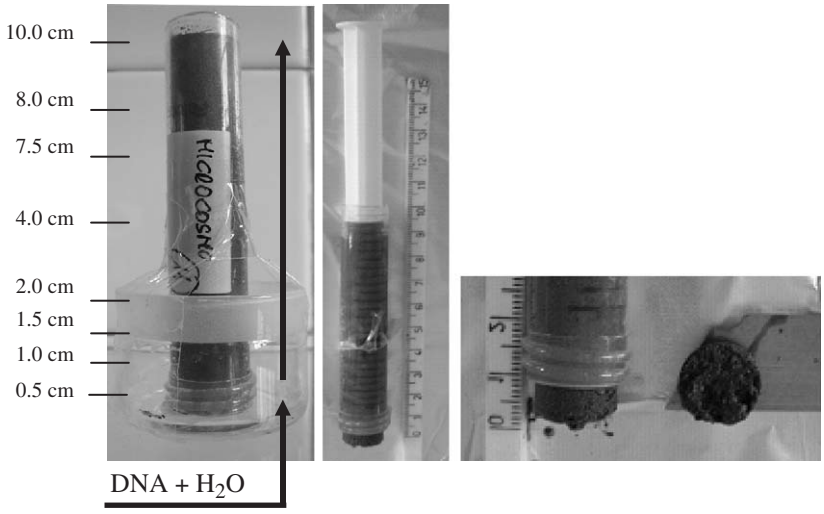

Fig. 1. Microcosm setup and soil-sampling procedure.

SPIN™ Filters), washed with a salt–ethanol solution and eluted in an appropriate volume of sterile ultrapure water.

DNA purity and concentration were checked and determined by spectrophotometric measurements at 280 and 260 nm (UV/VIS Lambda 2, Perkin Elmer) and by fluorimeter (DyNA-Quant™200, Hoefer).

2.5. PCR assays

The primers used to monitor the presence of the target sequence were 35S forward (GCTCCTACAAATGCCATCA) (Pietsch et al., 1997) and *nptII* reverse (GGT GGT CGA ATG GGC AGG TAG C) (Beck et al., 1982) and generated a fragment of 650 bp length.

The extracellular DNA (10 µl) was amplified with 40 µl reaction mixture containing 0.5 µl PolyTaq (5 U µl^{-1} Polymed, Firenze), 2 µl of each primer (10 mM), 5 µl reaction buffer (10 ×, Polymed, without $MgCl_2$), 7.5 µl $MgCl_2$ (10 mM, Polymed), 2 µl deoxynucleoside triphosphate mix (10 mM of each dNTP), 10 µl BSA (500 µg/ml) and 11 µl ultrapure water, in a final volume of 50 µl. The PCR was performed with a Perkin–Elmer 2400 thermocycler according to the following conditions: 95 °C for 2 min, followed by 25 cycles each at 95 °C for 60 s, 55 °C for 60 s and 72 °C for 90 s. In order to ensure a complete extension, a final step at 72 °C for 5 min was performed. The whole volume of each PCR product was subjected to horizontal electrophoresis on 1 × TAE (Tris-acetate buffer) agarose gel (1% w/v) containing 1:10,000 ethidium bromide (10 mg ml^{-1}), with appropriate DNA size standards (Mass Ruler™, DNA Ladder Mix Fermentas). Electrophoresis conditions were 100 V for 140 min. Negative controls consisted of the extracellular DNA extracted from the non-inoculated control microcosms and of sterile ultrapure water. The positive control was the genomic DNA from *A. calcoaceticus*.

The absence of the target gene construct (35S-*nptII*) in the control soil and inoculated microcosms was previously verified by PCR with the same primer set.

3. Results and discussion

Preliminary experiments were carried out to determine: (i) the minimum amount of the target DNA detectable in soil by PCR; (ii) the detection of the extracellular target DNA sequence in soil for a period of time sufficient to assess the vertical movement of DNA in soil columns.

The approach used allowed the visible monitoring of the water rise, the accurate and reproducible sampling of soil at definite column heights, and the specific target DNA detection without the risk of cross contamination.

The purity index of the extracted extracellular DNA was 1.8 as calculated by the spectrophotometric absorption ratio A_{260}/A_{280}.

3.1. Analysis of extracellular DNA in soil

One day after incubation, the amount of extracellular DNA in soil decreased to 66.6% of the initial amount. It remained constant for 3 days and decreased again to the 41% of the initial amount 20 days after incubation. However, the molecular weight of the extracellular DNA changed as shown by agarose gels, particularly after 10 and 20 days (Fig. 2). These changes can be attributed to degradation of DNA by microbial nucleases (Joenje and Venema, 1975; Suh et al., 1996). The target gene was detected using PCR followed by electrophoresis on agarose gels over a period of 10 days. However, after 20 days of incubation, no positive signals were detected by PCR, indicating that the amount of the target DNA was under the PCR detection limit and it was enzymatically degraded by soil nuclease activity and/or tightly bound to soil particles (England et al., 2005). After considering the above results, we chose a 3-day period for monitoring the DNA

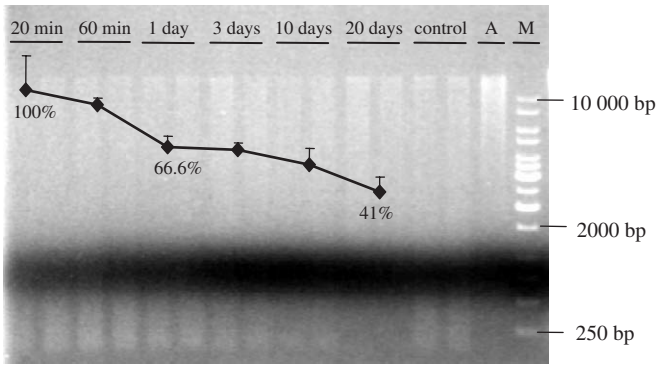

Fig. 2. Monitoring the persistence (on 0.8% agarose gel) of extracellular DNA as percentage (quantified by fluorometer) of the amount initially added to the soil. Twenty days: period of incubation; control: no-DNA spiked soil; A: genomic DNA from *A. calcoaceticus*; M: 1 kb DNA Ladder (Fermentas). Two of three replicates are shown.

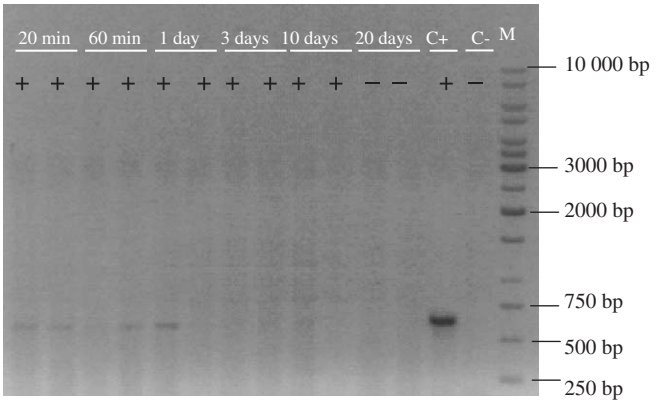

Fig. 3. PCR monitoring on agarose gel of the target sequence (35S-*nptII*) in DNA extracted from soil during the 20-day incubation period. ±: presence/absence of amplicons; C+: genomic DNA of *A. calcoaceticus*; C−: no-DNA inoculated soil; M: 1 kb DNA Ladder (Fermentas).

vertical advection by capillarity because the concentration of the target sequence would probably be significantly reduced during longer incubation times (Fig. 3).

3.2. Upward movement of extracellular DNA in soil columns

The DNA movement was evaluated by the presence or absence of the specific target DNA: 35S-*nptII* sequence. The control soils, dry or wetted, without any target DNA added did not show any amplification product (confirming the control in the DNA degradation experiment), indicating the absence of measurable 35S-*nptII* sequences in the soil. Therefore, the target fragment was useful as a marker for assessing the persistence and movement of the added extracellular bacterial DNA in the soil columns. In the soil columns with DNA addition, the water reached a visible level of 4 cm after 20 min, and PCR products were detected throughout the wetted soil (i.e., in 0.5, 1.0, 1.5, 2.0, 2.5 and 4.0 cm high samples). After 60 min, the entire soil column (10 cm) was wetted and the target sequence was detected up to a height of 7.5 cm (Fig. 4a). After 1 day of incubation and a second wetting with sterile water, amplicons of the target sequence were also found at heights of 8 and 10 cm. However, after 3 days of incubation and a third wetting, the marker sequence was only found at heights ranging from 0.5 to 4 cm (Fig. 4b).

The persistence of the marker sequence in the soil incubated under constant moisture (it was still detectable after 20 days) may be due to better contact between DNA and adsorbing soil colloids in the batch systems than in the soil column experiments. In addition, the DNA movement by capillarity might have improved the contact between extracellular DNA and soil nucleases.

The soil column results showed that the extracellular DNA added to the soil could move from the bottom to the top of the column by capillary forces within 1 day (Fig. 5). The capillary force is based on the superficial tension of the water meniscus, whose strength increases with the decrease in the diameter of the capillary tube. The soil physical characteristics required for capillarity are an adequate texture and a convenient soil structure for the formation of capillary tubes and a water gradient between the superficial and the lower soil layers (Lal, 2000). Among the constituents of the solid phase, clay and organic matter permit the aggregation of soil particles so as to form several small capillaries that connect the deeper soil layers with soil surface. Generally, water evaporation and plant root uptake create the necessary water gradient between the superficial and the lower soil layers. Thus, it may be hypothesized that water movement through the capillaries can raise DNA molecules in situ, if these molecules escape degradation by nucleases. Water movement by capillarity could possibly desorb DNA that is loosely adsorbed on soil particles surfaces. This phenomenon, called ''molecular combing'', has been previously described and discussed by Bensimon et al. (1995). After desorption, the DNA molecules can follow the movement of water by capillarity until the DNA molecules interact again with soil particles located in the higher soil. The distance between the desorption and the new DNA binding sites through the soil column depends on both the presence of DNA adsorbing colloids in soil and the speed of the water rise, which is inversely proportional to the capillary size. The desorption and re-adsorption of DNA by soil colloids, the presence of nucleases and the movement of water by capillarity can explain why the marker gene was located only at heights between 0.5 and 4 cm after 3 days. Both nucleases and DNA adsorbing colloids, probably present throughout the soil column, had not yet eliminated the marker gene between 0.5 and 4 cm due to its higher concentration than that below and above this height range. The target DNA was probably present in higher concentrations at the bottom of the column after the DNA solution addition. However, after the following two wetting cycles, the zone with highest DNA concentration

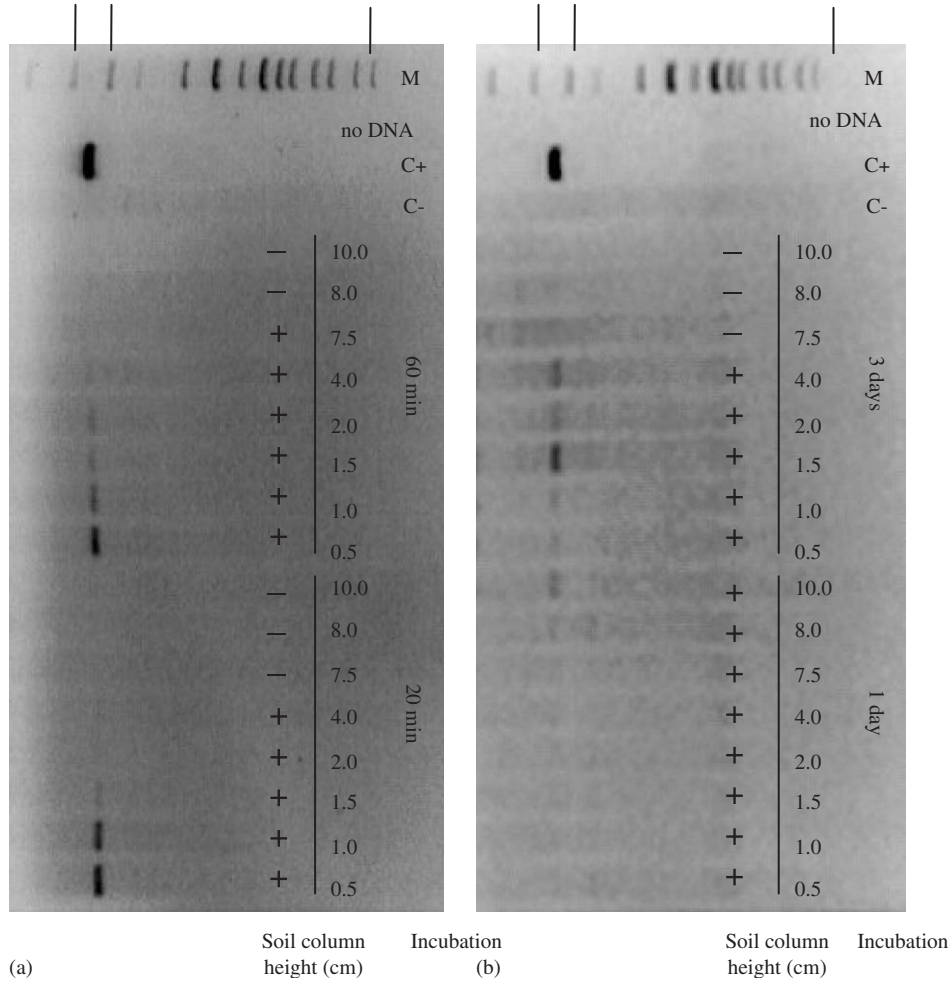

Fig. 4. Specific PCR detection of the 35S-*nptII* target sequence (650 bp) in soil columns. The monitoring was performed throughout the soil columns (from 0 to 10 cm) at 20 and 60 min (a) and 1 and 3 days (b) after the DNA inoculation. PCR products (50 μl) were analyzed by agarose gel electrophoresis. ±: presence/absence of amplicons; C−: no-DNA inoculated soil; C+: genomic DNA of *A. calcoaceticus*; no DNA: PCR reaction mix without template DNA; M: 1 kb DNA Ladder (Fermentas). The figure shows two representative gels with one of the three replicates.

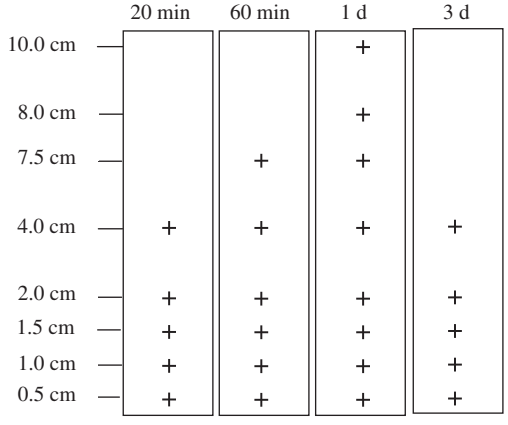

Fig. 5. Schematic summary of the column soil results. The presence of the specific PCR product (+) shows its upward movement throughout the soil column.

moved up due to the mechanisms discussed above (DNA desorption, DNA movement with water and re-adsorption of DNA by soil colloids).

4. Conclusions

We have demonstrated that DNA can reach higher soil layers by capillary rise and this has important implications in microbial ecology in soil, because it may allow horizontal gene exchange involving DNA released from bacteria living in a soil layer and bacteria inhabiting a different soil layer. This work also shows that dissolved DNA is mobile during capillary rise. The vertical advection by capillarity might be capable of desorbing loosely adsorbed DNA from soil colloids. In addition to DNA adsorption by soil colloids, nuclease and abiotic degradation also decreased the concentration of free extracellular DNA in the water phase.

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