



Biofilm resilience to desiccation in groundwater aquifers: A laboratory and field study



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HIGHLIGHTS

- Resilience of microbial communities in groundwater to desiccation is investigated.
- Comparisons of field and laboratory experiments are presented.
- Microbial communities can recover from temporary desiccation events.
- Field and laboratory experiments demonstrated resilience to temporary desiccation.
- Repeated prolonged desiccation may affect microbial communities.

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ABSTRACT

Groundwater is used as a precious resource for drinking water worldwide. Increasing anthropogenic activity is putting increasing pressure on groundwater resources. One impact of increased groundwater abstraction coupled with increasing dry weather events is the lowering of groundwater levels within aquifers. Biofilms within groundwater aquifers offer protection to the groundwater by removing contaminants entering the aquifer systems from land use activities. The study presented investigated the impact of desiccation events on the biofilms present in groundwater aquifers using field and laboratory experiments. In both field and laboratory experiments a reduction in enzyme activity (glucosidase, esterase and phosphatase) was seen during desiccation compared to wet controls. However, comparing all the data together no significant differences were seen between either wet or desiccated samples or between the start and end of the experiments. In both field and laboratory experiments enzyme activity recovered to start levels after return to wet conditions. The study shows that biofilms within groundwater systems are resilient and can withstand periods of desiccation (4 months).

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1. Introduction

New Zealand ranks in the top ten countries in the world for water quantity and quality (Scarsbrook and Pearson, 2012). The majority of this water is in the form of groundwater and provides a significant source (approximately 50%) of potable water and agricultural water uses e.g. irrigation and livestock drinking water (Oki and Kanae, 2006). Although water is abundant in New Zealand as a whole, increasing abstraction for potable water and agriculture is putting increasing pressure on these resources in certain areas of New Zealand. The water sources in New Zealand are not evenly spread and demand in drier areas, often in summer when natural recharge is at its lowest, is

putting groundwater systems under increasing pressure (Scarsbrook and Pearson, 2012). The competing needs of all users must be carefully managed to preserve both water quality (especially in regard to protection of public health) and quantity for future use.

Globally groundwater ecosystems are becoming under increasing pressure from anthropogenic activities and climatic changes (Danielopol et al., 2003; Green et al., 2011; Griebler and Lueders, 2009; Kundu and Mandal, 2009; Lerner and Harris, 2009; Van Der Zaan et al., 2010). One of the key concerns in New Zealand is the effect of increased abstraction on the ecosystem services delivered by the microbial populations living within the aquifers. These microbial communities exist primarily in protected structures (biofilms) on the gravel matrix. These biofilm communities are thought to carry out important ecosystem services such as biogeochemical cycling of nutrients and organic anthropogenic contaminants (Griebler and Lueders, 2009; Roling et al., 2001; Tischer et al., 2013; Waldron et al., 2009). There is evidence that microbial communities can adapt and remediate anthropogenic

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contamination entering the groundwater environment. Waldron et al. (2009) investigated the effect of heavy metal contamination on groundwater microbial diversity and function. Functional assessment in terms of the metal resistance, sulphate reduction, organic contaminant degradation, and carbon and nitrogen cycling were measured. Highly contaminated wells led to very low microbial diversity but function (activity) was high. There was evidence for both metal resistant and metal reducing microorganisms dominating the environment. An investigation into the effect of landfill leachate on microbial communities showed differences in the communities present upstream, within the leachate plume and downstream (Roling et al., 2001). There was evidence of microbial communities recovering from contamination with the disappearance in the plume and subsequent re-appearance of microorganisms downstream e.g. β Proteobacteria.

Research that is lacking presently is the effect of desiccation on these microbial communities and their metabolic functions and how they can recover when saturated conditions return. The communities are naturally adapted to seasonal fluctuations in groundwater height but may be adversely affected by over abstraction. The Zone of Intermittent Saturation (ZIS) describes the profile between the maximum and minimum groundwater tables that are sometimes saturated (below the water table) and sometimes unsaturated (above the water table). Increased abstraction will increase the depth of the ZIS profile and prolong the duration of desiccation. Monitoring of a number of wells in the Canterbury region has shown marked increases in the duration of desiccation events since irrigation was introduced to the region (Environment Canterbury, 2014). This will increase the number of biofilm communities exposed to seasonal desiccation stress and increase the duration of desiccation periods. As these ecosystems are used to remove contaminants and recycling of nutrients it is vital to understand what effect these stresses will have on their ability to self-remediate.

Measuring changes occurring in the groundwater microbial communities is inherently difficult given their location many metres below ground level. Traditionally, organisms have been cultured from the transient water community or less frequently from sediments (Aislabie et al., 2009; Brad et al., 2008). Currently, taking samples of groundwater communities is inherently difficult, expensive and there is a high potential for contamination of the sample. For porous aquifers it has been demonstrated that suspended prokaryotes (i.e. those found in transient groundwater and well water) do not accurately reflect the attached microbial populations (Elliott et al., 2010; Griebl and Lueders, 2009; Wilhartitz et al., 2009) which are attributed to providing the core ecosystem services (Griebl and Lueders, 2009). Griebl and Lueders (2009) provide a tabulated compilation of past research of transient groundwater and sediment habitats and the relative abundance of microorganisms (cells cm^{-3}) in each. The transient groundwater had lower abundance compared to sediments within these groundwater environments. Few studies have investigated the differences in transient groundwater and in situ groundwater sediments (Harvey et al., 1984; Hazen et al., 1991; Hirsch et al., 1992; Kölbl-Boelke and Hirsch, 1989; Kölbl-Boelke and Nehr Korn, 1992; Lehman et al., 2001; Lehman and O'Connell, 2002; Wilson et al., 1983). These studies showed a significant difference in the microbial populations (abundance) present in the transient groundwater and in situ sediments. Furthermore, there is a need to establish not only the abundance of organisms present but the activity of these communities within these systems (Williamson et al., 2012). Most studies addressing this question have taken samples from the environment and performed subsequent controlled incubation experiments (Griebl and Lueders, 2009). There is evidence that these controlled laboratory experiments have a tendency to overestimate the activity occurring compared to field samples (Steinweg et al., 2012).

Fluctuation of the water table in a nearby aquifer to the field site revealed that periods of desiccation are occurring for longer periods of time and desiccation of 4 months is commonplace since the introduction of irrigation in the area (Environment Canterbury, 2014). The research presented aimed to establish the effects of desiccation on microbial

abundance and enzymatic activity in groundwater. In addition, due to the difficulties in assessing biofilm activity in situ in groundwater environments, a comparison was made between in situ biofilms and laboratory controlled microcosm experiments. To test the effect of desiccation three treatments were tested: biofilms that remained saturated (below water table or groundwater passed over the bags throughout the experiment); biofilms that were removed from saturated environment (desiccated) for 4 months; and biofilms that were removed from saturated environment and then returned to saturated conditions (below water table or groundwater passed over the bags).

2. Methods

Based on our previous experiments an alternative to destructive sampling of the groundwater sediments was utilised (Williamson et al., 2012). In situ experiments were established at a specialised field site and in replicate laboratory microcosms to investigate microbial function after desiccation events were simulated.

A series of replicated laboratory microcosms and a field sampling site were established to measure the effects that those periods of intermittent saturation and desiccation have on the native microbial populations in groundwater aquifer communities.

2.1. Gravel biofilm establishment

The substratum used for biofilm development was fine greywacke gravel (average diameter: 2.8 mm and 1.5 g per gravel particle) with a particle density of $\sim 2.67 \text{ g/cm}^3$ (Hatherton and Leopard, 1964). Preparation of the gravel has been previously described (Williamson et al., 2012). Briefly, the gravel (approximately 200 g) was placed in gravel bags (diameter 90 mm) made from nylon with a mesh size of $37.9 \pm 6.0 \mu\text{m}$, which would exclude some but not all protozoa to optimise biofilm establishment. The finished bags were autoclaved twice for 1 h at $121 \text{ }^\circ\text{C}$, with drying at $65 \text{ }^\circ\text{C}$ between, before final drying at $65 \text{ }^\circ\text{C}$ for 2 days. The bags were then either placed in the ZIS field site (Section 2.2) in positions below the water table i.e. saturated or packed into prepared laboratory microcosms (Section 2.3). A biofilm population (5 months for field site and 10 months for microcosms) was allowed to establish based on previous research (data not shown). After biofilm establishment had occurred, the baseline biological activity using enzyme and biomass assays were determined.

The following test conditions were applied to mimic the annual cycle of desiccation and saturation for both field and laboratory experiments:

1. Saturated (wet) – for the whole experimental period,
2. Saturated and desiccated (wet then dry) – mimicking desiccation conditions: biofilm bags raised above the water table at the field site or groundwater flow stopped in the microcosms for a period of time (4 months) and the microcosms drained,
3. Saturated, desiccated and saturated (wet then dry and then wet) – after desiccation conditions (as per number 2 above): Biofilm bags were replaced below the water table at the field site or groundwater flow returned to the microcosms.

2.2. Field site

The field site selected was located on the Canterbury Plains, New Zealand, in an area over an unconfined alluvial aquifer. This area has a high number of dairy farms which have been converted from traditional cropping and sheep and beef farming. The field site was prepared by drilling a sampling well (1.5 m in diameter) to a depth of 6 metres (m). The well was encased in high strength polyethylene and had ladder and winch access to permit sampling. The encased well was covered by a shipping container to provide protection from wandering stock, sunlight and adverse weather. The aquifer materials encountered over this depth range (0–6 m below ground level (bgl)) included sandy

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