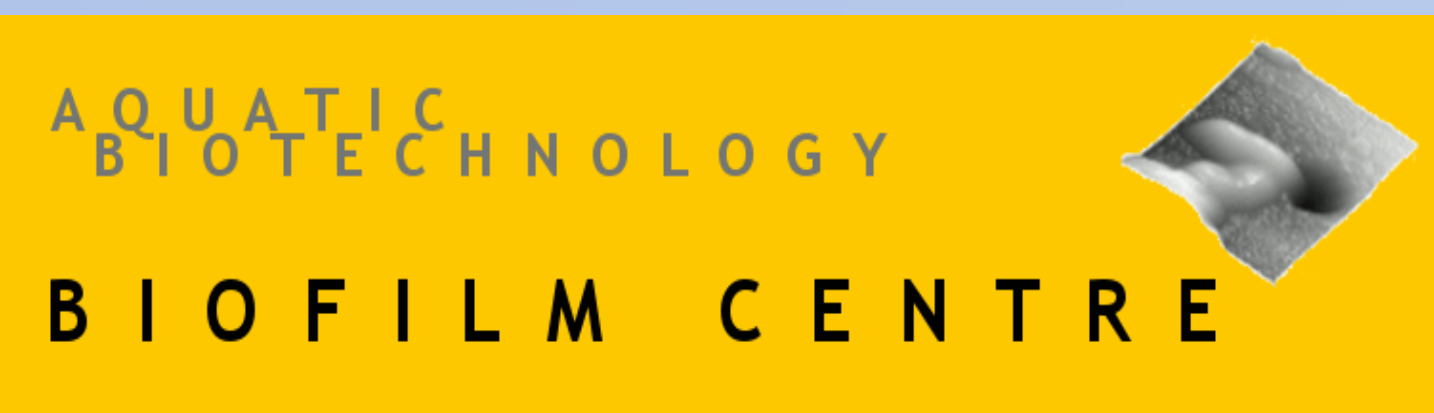


Microbial community composition on lignite before and after the addition of phosphate mining wastes

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Introduction

Field and laboratory work to reduce sulfide oxidation in pyritic waste rock using various phosphate formulations including phosphate mining wastes (Natural Phosphate Rock, NPR) has been an intensive focus since the mid nineteen eighties [1-4]. The noted reductions in sulfide oxidation were thought to be the result of the various iron-phosphate precipitates. However in 2003, Jones [5] refuted the effectiveness of an iron phosphate coating confirming the presence of microbes underneath the coating on arsenopyrite. In 2005, Mudder et al. [6] summarized 5 year laboratory and field tests using thiocyanate as biocide in combination with NPR and commercial fertilizers. They proposed a combined biotic–abiotic effect on the initial reduction of acid generation. However, this effect was short-lived. Testing local NPR and commercial fertilizer on sulfidic waste rock in Australia, Mauric and Lottermoser [7] found that phosphate was ineffective in reducing sulfide oxidation. In 1991 long-term tests in various sulfidic wastes were set up in Canada using NPR by Boojum Research Ltd. Samples were recovered after a period of 3 to 5 years. After extensive exposure to oxidizing conditions, effluents with NPR produced low acidity [8]. The surfaces of sulfidic waste rock from one of the experiments where NPR was added, were inspected with SEM/EDX [9]. An organic biofilm was noted covering the mineral surface [10]. Only traces of iron phosphate precipitates were found. Rock surfaces from the experiment after being stored outdoors in plastic containers for 11 years still showed the presence of the biofilms [11]. An hypothesis of the possible reactions occurring after addition of NPR to acid-generating waste rocks was formulated [12]. Previous work emphasized the effluent improvements and investigated the coatings on the mineral surfaces. For the first time, we present a description, including an enumeration of the microbial communities found associated with German lignite, in the presence and absence of NPR, and under sterile and microbe-inoculated conditions using a column experiment.

A long lasting effect:

Improvement of effluent water quality (270 days after NPR addition)



Column	pH	E _H [mV]	Conductivity [mS]
B1 (-NPR)	1.6	686	12.0
B3 (-NPR)	1.6	687	16.9
B2 (+NPR)	2.3	648	5.6
B4 (+NPR)	2.4	616	4.6

Materials and methods

Column Setup Bioleaching columns were set up in sterile 100-mL syringes (PP, Pentaferte[®]) using 1 g creased glass wool to prevent clogging. After filling the columns with 100 g lignite (1-2 % pyrite), 1 g of additional fine ground pyrite (>100 µm) from Bala Mare, Romania was added. For microscopic observation of pyrite colonization 10 pyrite coupons (2x2x1 mm) were placed in the centre of the columns. For sterile control columns, the lignite was autoclaved three times for 1 hour at 121 °C, 1.5 atm. The biological treatment columns were inoculated with 10⁸ cells/g lignite-cultured microbes using an enrichment culture from Rio Agrio, Argentina, maintained at 30 °C using pyrite as sole energy source. Two and four weeks after inoculation, in two out of four columns (one each sterile and biological), 1.2 g of finely ground NPR (<36 µm grain size) was dispersed evenly on top of the lignite. Hence, four different column treatments were tested in duplicate, sterile (S) and biological treatment (B) columns each with (+NPR) and without addition of NPR (-NPR).

Sampling of effluent and column material The columns were placed vertically in a 20 ml beaker to collect the effluent and incubated at 30 °C. To initiate bioleaching, 10 mL basal salt solution (pH 1.8) after [13] the columns were flushed through the columns over 3 days to remove old oxidation products. Before the NPR addition and after 2, 4 and 10 weeks 1 g wet weight lignite was removed from the centre of the columns to quantify acidophilic iron-oxidizing, acidophilic sulfur-oxidizing prokaryotes, acidophilic and neutrophilic heterotrophs with the most probable number (MPN) technique [14]. Post NPR addition, rain water was used as leach medium for B columns at intervals of 2-3 days while sterile-filtered (0.22 µm) rain water was used for S columns. After 56 days the sampling intervals were increased to one week. The recovered effluent volume was about 5 mL after 20 min. Measurements of pH (Mettler Toledo, InLab[®], Easy Electrode), E_H (Mettler Toledo, InLab[®], 501/170 Electrode) and conductivity (Windaas, WinLab[®] meter) was carried out immediately after flushing. The samples were then stored at -20 °C.

Quantification of selected groups of microorganisms by MPN The basal salt solution for cultivation of acidophilic microorganisms after [9] was used. This medium with 4 g/L ferrous iron and the same medium (adjusted to pH 2.5) with 5-fold phosphate content and 1 % (w/v) elemental sulfur powder (Merck[®]) were used for cultivation of iron- and sulfur-oxidizing acidophiles, respectively. The same basal medium with 0.5 g/L glucose and 0.02 % yeast extract was used for cultivation of acidophilic, heterotrophic microorganisms. Neutrophilic heterotrophs were quantified using R2A-agar plates.

Results

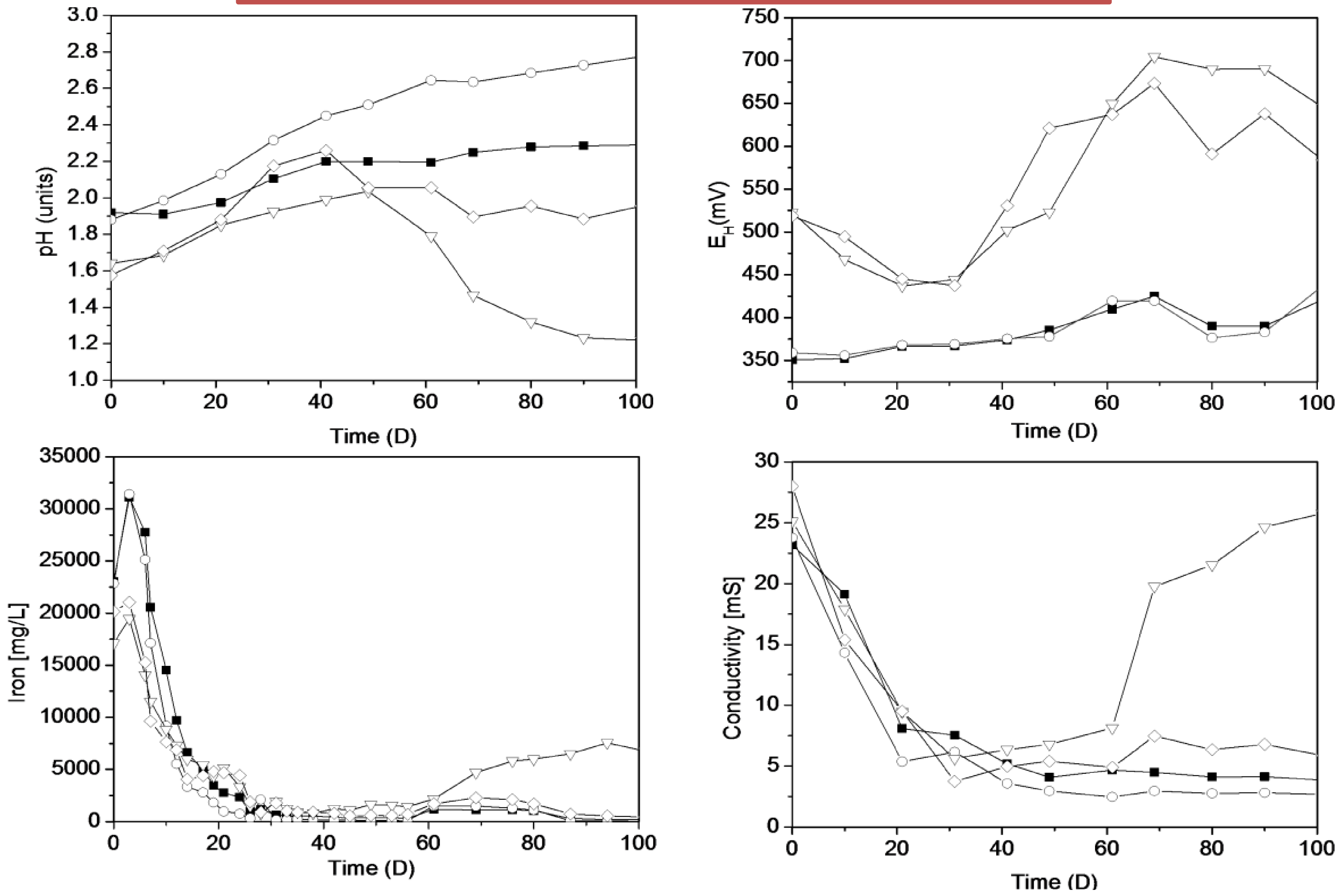


Fig. 1. pH, E_H, conductivity and [iron] in the effluents from column leaching assays. Mean values from duplicates; ○ Sterile with NPR addition, ■ Sterile without NPR, ◇ biological with NPR and ▽ biological without NPR.

In the absence of NPR, bioleaching started after day 45 with the release of ferric iron, increases in redox potential and electrical conductivity values, and decreasing pH values. The addition of NPR inhibited bioleaching resulting in an improved effluent quality. The development of the percentage distribution of 4 microbial groups quantified by MPN is plotted in Fig. 2. No change of the microbial community composition occurred without addition of NPR, while its addition strongly proliferated neutrophilic heterotrophs. NPR led concurrently to a reduction in the number of acidophilic iron oxidizers. Four weeks after NPR addition the columns were dominated by neutrophilic heterotrophs (90 %), while without NPR, iron-oxidizing acidophiles accounted for 99 % of the microbial community.

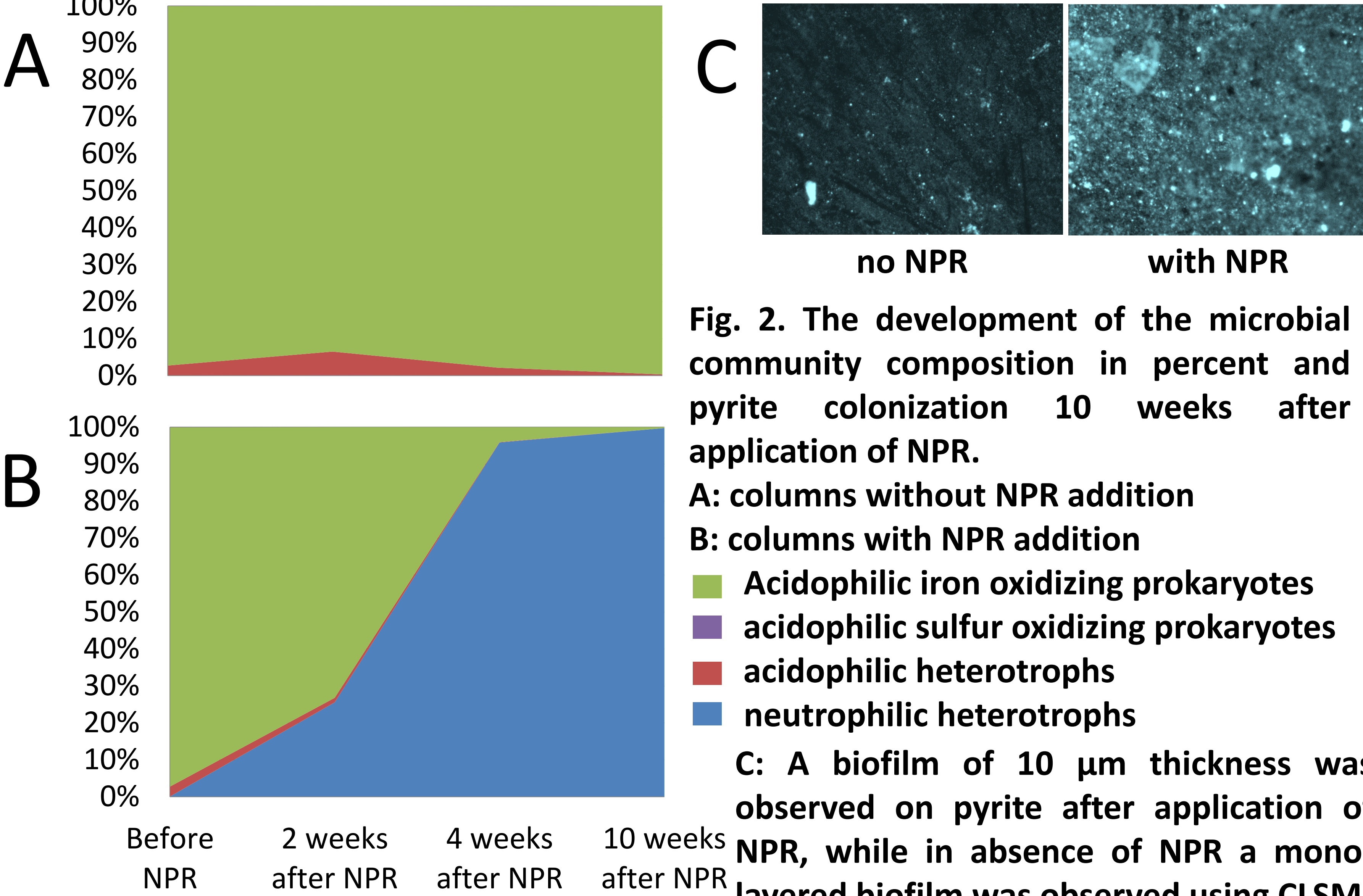


Fig. 2. The development of the microbial community composition in percent and pyrite colonization 10 weeks after application of NPR.

A: columns without NPR addition

B: columns with NPR addition

- Acidophilic iron oxidizing prokaryotes
- acidophilic sulfur oxidizing prokaryotes
- acidophilic heterotrophs
- neutrophilic heterotrophs

C: A biofilm of 10 µm thickness was observed on pyrite after application of NPR, while in absence of NPR a mono-layered biofilm was observed using CLSM.

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