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Biostimulation is a valuable tool to assess pesticide biodegradation capacity of groundwater microorganisms

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ABSTRACT

Groundwater is the main source for drinking water production globally. Groundwater unfortunately can contain micropollutants (MPs) such as pesticides and/or pesticide metabolites. Biological remediation of MPs in groundwater requires an understanding of natural biodegradation capacity and the conditions required to stimulate biodegradation activity. Thus, biostimulation experiments are a valuable tool to assess pesticide biodegradation capacity of field microorganisms. To this end, groundwater samples were collected at a drinking water abstraction aquifer at two locations, five different depths. Biodegradation of the MPs BAM, MCPP and 2,4-D was assessed in microcosms with groundwater samples, either without amendment, or amended with electron acceptor (nitrate or oxygen) and/or carbon substrate (dissolved organic carbon (DOC)). Oxygen + DOC was the most successful amendment resulting in complete biodegradation of 2,4-D in all microcosms after 42 days. DOC was most likely used as a growth substrate that enhanced co-metabolic 2,4-D degradation with oxygen as electron acceptor. Different biodegradation rates were observed per groundwater sample. Overall, microorganisms from the shallow aquifer had faster biodegradation rates than those from the deep aquifer. Higher microbial activity was also observed in terms of CO_2 production in the microcosms with shallow groundwater. Our results seem to indicate that shallow groundwater contains more active microorganisms, possibly due to their exposure to higher concentrations of both DOC and MPs. Understanding field biodegradation capacity is a key step towards developing further bioremediation-based technologies. Our results show that biostimulation has real potential as a technology for remediating MPs in aquifers in order to ensure safe drinking production.

1. Introduction

Groundwater is the major resource globally used for drinking water production (Ekins et al., 2019). In the Netherlands, the groundwater quality is monitored to ensure safe drinking water production. Monitoring data shows that groundwater quality is threatened by the presences of micropollutants (MPs) (Loos et al., 2010). Pesticides are among the most commonly encountered MPs, as they are widely used in agriculture. In 2018, the Food and Agriculture Organization of the United Nations (FAO) determined that an average of 8.79 kg of pesticides per hectare of cropland were used in The Netherlands (FAO, 2018). A recent study examining drinking water-abstraction areas covering groundwater and surface water bodies in the Netherlands found pesticides and/or metabolites in 150 out of 226 samples (Sjerps et al., 2019). These studies demonstrate the urgency of finding solutions to remove pesticides from aquifers and guarantee safe drinking water production.

Biological remediation has been studied previously for pesticide polluted environments such as agricultural soil (Bers et al., 2013; Cheyns et al., 2012). Lately, biodegradation has also been studied as a remediation alternative for groundwater (Greskowiak et al., 2017; Zhang et al., 2017). Natural attenuation is one of the best strategies for addressing groundwater contamination, as its a natural process with low side-effects on the aquifer (Balderacchi et al., 2013; Gavrilescu et al., 2015; Meckenstock et al., 2015). However, this solution may not be ideal in cases where intrinsic in-situ biodegradation processes are limited due to (1) lack of electron acceptors, (2) lack of growth substrate, (3) absence of degrading microorganisms (Helbling, 2015; Scow and Hicks, 2005). Groundwater is characterized as an anaerobic oligotrophic environment which can restrict microbial activity (Egli, 2010; Benner et al., 2013; Kolvenbach et al., 2014). Aquifers become increasingly

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anaerobic with depth, as electron acceptors are sequentially consumed (McMahon and Chapelle, 2008). DOC quantity and quality also decreases with depth in the aquifer (Shen et al., 2015). Thus, the general microbial population size and activity in aquifers is low, which can limit biodegradation (Helbling, 2015; Helbling et al., 2014).

Limited intrinsic biodegradation activity can be overcome by supplementing for instance electron acceptors, carbon or degrading microorganisms. Redox conditions determine microbial MPs degradation activity, as has been shown in laboratory experiments (Barbieri et al., 2011; Ghattas et al., 2017; He et al., 2017). Thus, traditional bioremediation strategies often include amendments with electron acceptors, such as oxygen or nitrate (Cunningham et al., 2001). Oxygen is the best electron acceptor, and thus useful for MPs biodegradation processes (Schmidt et al., 2017). Nitrate addition can also enhance biomass production, which can result in some MP biodegradation (Holtze, 2011; Schreiber and Bahr, 2002). Carbon substrates are also necessary for growth and activity. Carbon limitation in oligotrophic groundwater can be overcome by addition of dissolved organic carbon (DOC), with positive results on the biodegradation of certain MPs during groundwater recharge or in column experiments (Hoppe-Jones et al., 2012; Horemans et al., 2013; Luo et al., 2019). Such amendments thus overcome limitations to intrinsic groundwater biodegradation activity, to create conditions conducive to biodegradation. A prerequisite to these approaches is the presence of intrinsic biodegradation capacity in groundwater.

In this study, we aimed at assessing MPs biodegradation capacity of microbial communities in groundwater of a drinking water abstraction area towards one pesticide metabolite and two pesticides: 2,6-dichlorobenzamide (BAM), mecoprop-p (MCPP) and 2,4-dichlorophenoxyacetic acid (2,4-D). Groundwater samples were taken from two monitoring wells located at the north-east of The Netherlands, at five different depths ranging from 13 to 54 m below ground level. Groundwater composition varied with depth, including redox conditions and natural DOC concentration. Microcosm experiments were used to study the biodegradation capacity of field microorganisms under the influence of four different amendments: nitrate + DOC, oxygen + DOC, oxygen, and DOC. Our results provide evidence that there is 2,4-D biodegradation capacity in the field, and that biostimulation has potential for application as an in-situ remediation technology.

2. Materials and methods

2.1. Chemicals and reagents

The MPs analytical standards 2,6-dichlorobenzamide (BAM), mecoprop-p (MCPP) and 2,4-dichlorophenoxyacetic acid (2,4-D), were purchased from Sigma-Aldrich (USA). A stock MPs solution was prepared by adding the three MPs to MilliQ (MQ) water to a final concentration of 100 mg/L. The stock nitrate solution was prepared by dissolving $NaNO_3$ (Sigma-Aldrich) in MQ water to achieve a 400 mM concentration. Green compost (Van Iersel Compost, The Netherlands), with a composition of 50% screened wood, 25% grass litter, and 25% leaf litter was used for DOC extraction as described in Luo et al. (2019). DOC concentration of the stock solution was determined by measuring NPOC (non-purgeable organic carbon) with a TOC-L_{CPH} analyser with ASI-L autosampler (Shimadzu, Japan).

2.2. Groundwater sampling

The groundwater samples were taken from two monitoring wells at a drinking water abstraction location (22 and 23) at five different depths per well. The wells are located in an agricultural area in the northeast of the Netherlands, around 500 m apart from each other. Well 22 is further upstream from the extraction location and adjacent to a canal while well 23 is rather isolated and closer to the extraction location. Further details on the site description can be found in Aldas-Vargas et al. (2019). The chemical groundwater composition from both wells on the day of sampling is provided in Table 1. The data presented was facilitated from a project partner.

Before sample collection, the well tube was flushed by extracting and discarding at least three times the well tube volume. Turbidity was continuously measured while the water was being pumped, and the samples were taken after turbidity stabilized below 1 NTU. Samples were collected in 1 L amber glass bottles pre-washed with ethanol. The bottles were filled to the top to prevent oxygen intrusion and stored at 4 °C over-night before the microcosms were set-up.

2.3. Biostimulation microcosms set-up

The intrinsic biodegradation capacity of the groundwater microbial community was assessed in microcosm biodegradation experiments in 200 mL serum bottles. Additionally, MQ water was used as a control to determine if biodegradation activity comes from groundwater or from the DOC solution. Serum bottles were filled with 150 mL groundwater or MQ. Nitrate, DOC and MPs solutions were added to the bottles according to Table 2. During this procedure, the water, nitrate, DOC and MPs solutions were bubbled with nitrogen gas to ensure no addition of oxygen into anaerobic microcosms. A total of 156 serum bottles were used to study five different conditions in triplicate for all the groundwater and MQ samples. Final liquid volume per microcosm was maximum 166 mL. Bottles were sealed with viton stoppers and aluminium caps prior to headspace exchange using the gas exchanger. The headspace of each bottle was filled with either atmospheric pressurized air for aerobic conditions or nitrogen for anaerobic conditions and set to 1.50 bar. The bottles were covered with aluminium foil to prevent photodegradation and were kept on shakers set at 120 rpm and 20 °C for 213 days.

Liquid and headspace gas samples were taken periodically as displayed in Table 3. The liquid samples (2 mL) were collected with a syringe for measuring MPs and were centrifuged at 15.000 rpm for 10 min and stored at minus 20 °C before analysis. BAM, MCPP and 2,4-D were quantified with a UPLC-DAD (diode array detection) system. The eluents used for this method were ultra-pure water with 0.1% formic acid (FA) and acetonitrile with 0.1% FA. The detection limit is 0.02 mg/L, and details of the method can be found in Luo et al. (2019).

Pressure was measured using a GMH3150 meter (Greisinger Electronics, Germany) and gas samples from the headspace (2 mL) were taken from each microcosm using a syringe. The gas samples were measured directly after sampling. Afterwards, headspace exchange was performed on the microcosms with pressure lower than 1.5 bar. Concentration of the gasses O_2, CO_2, N_2, and CH_4 in the headspace of the bottles was measured with GC (GC-2010, Shimadzu). Details of the method can be found in de Wilt et al. (2018). The gas composition initially obtained in percentages, was recalculated to mmol by the use of the ideal gas law and headspace pressure. Chromeleon software (Thermo Fisher Scientific, USA) was used for analysis of the data from both liquid and gas chromatography.

3. Results and discussion

3.1. Intrinsic biodegradation capacity in the field

The groundwater microbial biodegradation capacity for BAM, MCPP and 2,4-D was evaluated using groundwater samples from five different depths of two monitoring wells. The blank sample, with no amendments showed no BAM, MCPP or 2,4-D biodegradation over 213 days (Fig. 1), indicating that field conditions are not conducive to MP biodegradation activity within the incubation period. Furthermore, we assessed the impact of four different amendments on the stimulation of biodegradation activity: (1) nitrate + DOC, (2) oxygen + DOC, (3) oxygen, and (4) DOC. The pesticide 2,4-D was degraded in all groundwaters stimulated with oxygen + DOC (Fig. 1). The other amendments, such as: nitrate + DOC, oxygen alone and DOC alone, did not result in 2,4-D biodegradation during 213 days of incubation. BAM and MCPP were

Table 1
Chemical composition of wells 22 and 23 at field conditions.

		22–1	22–2	22–3	22–4	22–5	23–1	23–2	23–3	23–4	23–5
Depth	**(m)**	13.0	21.5	28.0	40.0	46.0	13.5	27.0	37.0	47.0	54.0
Temp	**(°C)**	13.34	11.62	11.39	10.86	10.69	10.20	10.37	10.39	10.36	10.39
pH		6.91	6.86	6.83	7.63	7.51	5.64	5.29	5.61	6.77	6.91
NO_3^-	**(mg/L)**	<0.5	<0.5	<0.5	<0.5	<0.5	52	1.5	54	<0.5	<0.5
Fe (II)	**(mg/L)**	0.46	7.9	6.8	1.1	1.3	0.03	0.17	1.8	7.8	8.4
SO_4^-	**(mg/L)**	31	4.1	<0.5	34	81	29	77	170	100	84
DOC	**(mg C/L)**	8.3	8.9	9.2	4.8	3.8	6.8	5.4	3.4	2.7	3.6
BAM	**(ug/L)**	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.53	<0.5
MCPP	**(ug/L)**	<0.01	0.012	0.036	<00.1	<0.01	<0.01	<0.01	<0.01	<0.01	0.3
2,4-D	**(ug/L)**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 2
Set-up of biostimulation experiment. Five sets of amendments were applied to 10 different groundwater samples (22 and 23 depths 1–5). Triplicates were used for all sets.

Sample	Amendments	MPs (mg/L)[a]	DOC (mg C/L)[b]	Nitrate (mg/L)[c]	Headspace (1.5 bar)
Groundwater (wells 22 and 23)	Nitrate + DOC	1	+15	30	Nitrogen
	Oxygen + DOC	1	+15	–	Pressurized air
	Oxygen	1	–	–	Pressurized air
	DOC	1	+15	–	Nitrogen
	Blank	1	–	–	Nitrogen
Control (no groundwater)	Nitrate + DOC	1	15	30	Nitrogen
	Oxygen + DOC	1	15	–	Pressurized air

[a] The spiking solution of MPs contained BAM, MCPP and 2,4-D, each at a concentration of 1 mg/L.
[b] DOC was present in some of the groundwater samples, but equal amounts were added to all bottles.
[c] Nitrate was present in groundwater samples 23–1, 23–2 and 23–3 (Table 1), so no extra nitrate was added to these bottles.

not biodegraded in any of the groundwater samples, regardless of the different amendments (Figures S1 and S2).

Complete biodegradation of 2,4-D occurred in all the groundwater samples amended with oxygen + DOC after 42 days (Fig. 1). Our results demonstrate that DOC was needed for 2,4-D biodegradation, but also that DOC can only support biodegradation when there is oxygen present. Thus, we can conclude that 2,4-D biodegradation in our experimental set-up is an aerobic, co-metabolic process, where DOC is required for biodegradation activity. This seems to indicate that low concentrations of MPs are insufficient as carbon and energy source to initiate microbial growth, and an additional carbon substrate is necessary (Kennes-Veiga et al., 2020; Yang et al., 2018). There is also the possibility of microorganisms in the DOC solution to have an effect on MPs degradation, this topic will be discussed in Section 4.2.

Furthermore, our results indicate that DOC quality, rather than quantity, determines biodegradation activity. The DOC that was added to the bottles as part of the experiment originates from green compost and was freshly extracted before starting the experiment (Section 2.3). Our results confirm that additional easily degradable carbon substrates such as DOC derived from compost stimulate MP biodegradation activity in groundwater microbial communities. DOC is naturally present in groundwater at a similar concentration to the quantity amended in the current experiment. The DOC is consumed during infiltration of water into the aquifer, resulting in higher DOC concentrations in the shallow part of the aquifer compared to the deeper part (Table 1). For instance, we spiked 15 mg C/L into our experiments, which is similar to groundwater (Tables 1 and 2). Upon addition of only oxygen (Fig. 1A) or DOC (Fig. 1B) in shallow samples, limited 2,4-D degradation occasionally occurs in individual microcosms, resulting in the relative large error bars in the triplicate microcosm results. A possible explanation for the oxygen amendment results could be that the naturally present organic matter in the groundwater triggers some degradation in some but not all individual microcosms, but degradation stops after the reactive part of the organic substrate is exhausted upon biological oxidation. Previous research indicates that DOC quality influences 2,4-D biodegradation activity and found that DOC originating from compost supported higher biodegradation rates than DOC from groundwater (Luo et al., 2019). In the current study, we also found that DOC quality determined microbial MP biodegradation activity.

There are different mechanisms by which DOC can support biodegradation: (1) DOC acts as a growth substrate, providing microorganisms a carbon and energy source (Bowen et al., 2009; Horemans et al., 2017); (2) DOC acts as an electron shuttle, by being reduced or oxidized and liberating electrons (Aeschbacher et al, 2010, 2011; Lovley, 2000; Lovley et al., 1996) and (3) DOC acts as a structural analogue to MPs, by stimulating microorganisms to produce enzymes able to metabolize DOC and MPs (Hoppe-Jones et al., 2012). We hypothesize that in the current experiment, DOC was consumed as a growth substrate in the presence of oxygen. DOC amendment did not support 2,4-D biodegradation under anaerobic conditions (Fig. 1). Thus, only in presence of oxygen, DOC is able to support microbial activity and to facilitate MPs degradation.

Biodegradation of BAM and MCPP was not observed (Figures S2 and S3). These compounds are known to be recalcitrant MPs, that are less biodegradable than 2,4-D (Albrechtsen et al., 2001; Torang et al., 2003). Despite the literature studies where BAM and MCPP are biodegraded in both aerobic and anaerobic conditions (Buss et al., 2006; Ellegaard-Jensen et al., 2017; Frková et al., 2016), the groundwater microbial communities in our study either did not have biodegradation capacity for these compounds or required other amendments to support biodegradation activity (Figures S2 and S3). One explanation for this finding could be that BAM and MCPP degraders were not present in the groundwater samples used in our study, but that they rather form biofilms that are attached to the sediment. Another possibility is that BAM and MCPP can be co-metabolically degraded by the addition of other co-factors that were not provided in the current experiment. We

Table 3
Sampling days for the microcosms from groundwater samples (22 and 23 depths 1–5) and MQ samples.

	Well 22 (all microcosms)								Well 23 (all microcosms) and MQ (all microcosms)							
Liquid sample	0	14	42	56	85	109	146	210	0	14	42	56	84	111	148	212
Gas sample	7	14	34	50	91	111	148	213	7	14	34	50	91	111	148	213

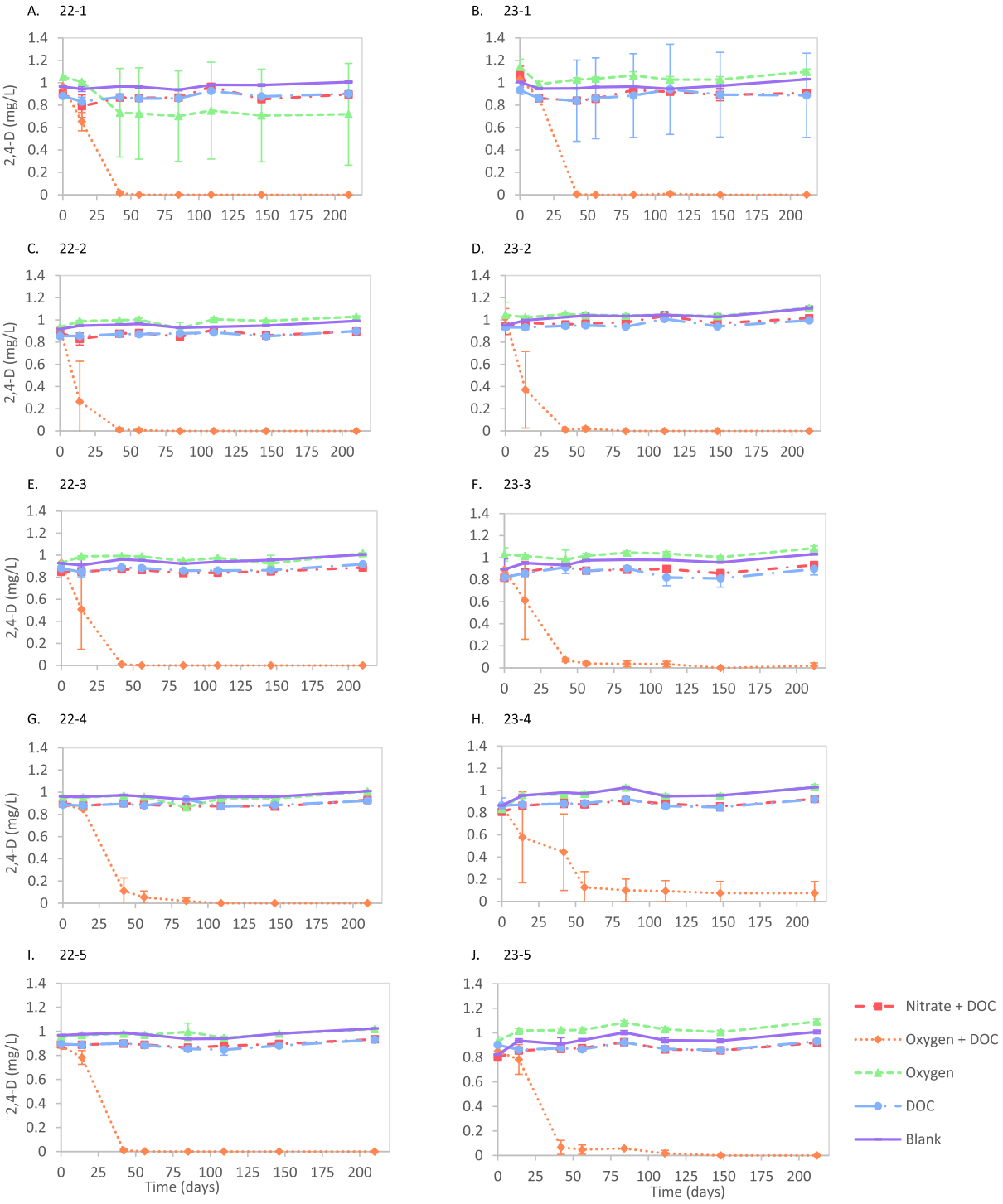

Fig. 1. 2,4-D concentration changes in time for four different amendments and the blank. Groundwater samples from wells 22, 23 at five different depths (Table 1). Lines represent different amendments and error bars correspond to three experimental bottles.

observed our amendments created suitable conditions to activate heterotrophs, but BAM or MCPP degradation was not observed. Moreover, the presence of a more easily degradable MP (i.e., 2,4-D) could also prevent microorganisms from degrading other MPs such as BAM and MCPP. Therefore, for future studies we suggest exploring biodegradation capacity of groundwater sediments and examining other amendments than those studied here.

3.2. *Differences in biodegradation rates per groundwater depth*

The 2,4-D biodegradation capacity is an intrinsic characteristic of groundwater microorganisms that changes per well and per depth. 2,4-D biodegradation rates in the presence of oxygen + DOC depend on the location of the sample (the well location) and the depth (Fig. 2). Overall, shallower groundwater showed higher 2,4-D biodegradation rates than deeper groundwater. For well 22, the shallow groundwater has the fastest 2,4-D degradation rate, with 22–2>22–1>22-3. Groundwater from 23-2 and 23-3 showed rapid biodegradation of 2,4-D, however

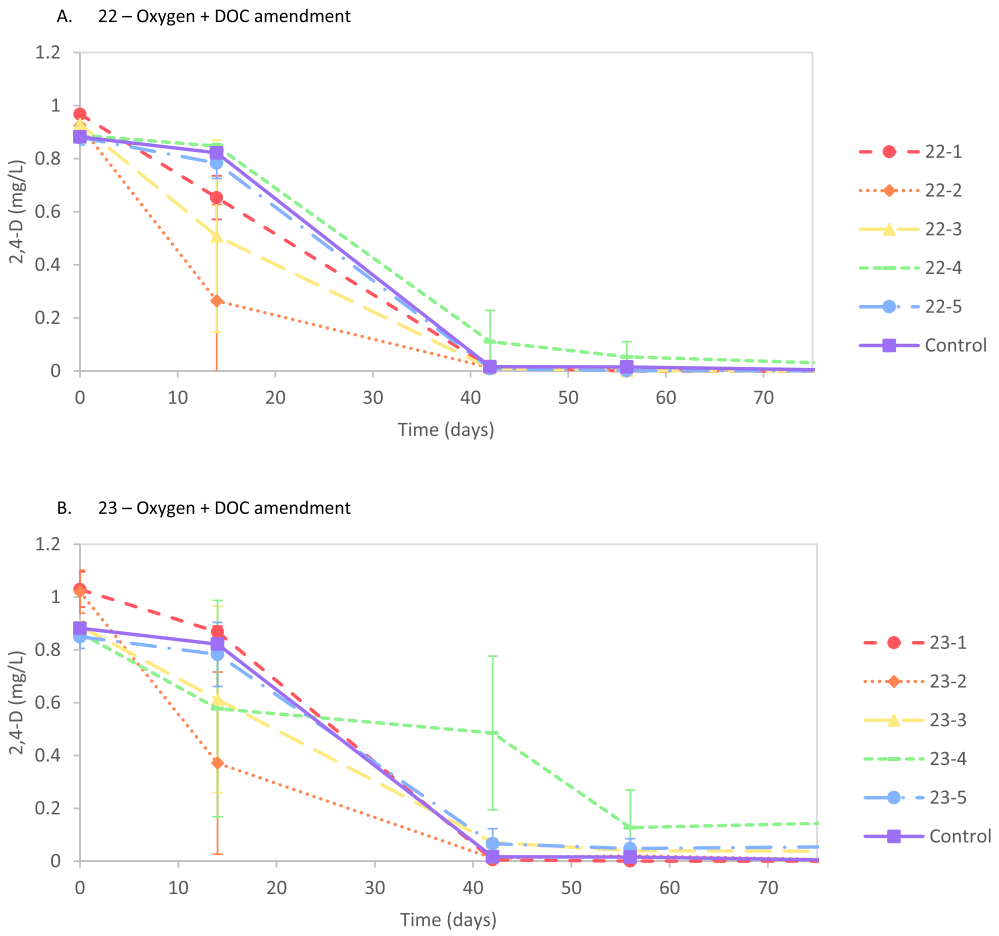

Fig. 2. 2,4-D degradation from day 0–75. Triplicate samples from well 22 (A) and well 23 (B) at different depths amended with Oxygen + DOC. The individual microcosm results can be found in Figure S3.

groundwater from 23-1 did not degrade as fast as the other shallow well-samples. Furthermore, the control without groundwater amended with oxygen + DOC also degraded 2,4-D, but at a slower rate than some groundwater samples (Fig. 2). This indicates that microorganisms in the DOC solution most likely contributed to 2,4-D biodegradation, but that groundwater microbial communities have a higher contribution to the observed degradation rates. These observations demonstrate that 2,4-D biodegradation activity depends on the microbial communities present in the groundwater, which in turn is determined by the intrinsic environmental conditions in the field that change per well location and per depth.

Microbial composition is a determining factor in MPs biodegradation. In a previous field study, the microbial communities from well 22 and 23 were studied. In well 22, more than 50% of the phyla corresponded to Proteobacteria, while in well 23, 24% was Proteobacteria and 21% Omnitrophica (Aldas-Vargas et al., 2019). We observed that the microbial composition in shallow groundwater from wells 22 and 23 differed from deep well samples (Aldas-Vargas et al., 2019). Shallow microbial communities in wells 22 and 23 were different with respect to each well, while microbial composition from deep well samples was similar between both wells. Moreover, the total bacteria concentration in this field samples was higher for 22–1, 22–2, 22–3, 23–2 and 23–3, as compared to other groundwater samples (Aldas-Vargas et al., 2019). Despite the fact that these groundwater samples were collected at different times, the amount of bacteria previously reported in the samples corresponds well with the observed microbial activity in the current study. It should be noted that assuming homogeneous groundwater flow, the upper part of the aquifer is probably exposed to higher MPs concentrations and in principle to rather fresh DOC (Jardine et al., 2006; Shen et al., 2015). In the case of well 22, there is also the possibility that some MPs can be horizontally infiltrated from the adjacent canal. For well 23, MPs could be carried in the deep aquifer by horizontal groundwater flow rather than from shallow filters. However, in most cases DOC as well as MPs travel through shallow groundwater before reaching deeper parts of the aquifer, indicating that shallow groundwater can be exposed to higher concentrations and for longer periods to MPs and DOC. This exposure can result in more active biodegradation due to microbial adaptation processes (Poursat et al., 2020).

In order to evaluate the microbial activity, CO_2 production was monitored. The cumulative CO_2 production was calculated per microcosm and the values of the triplicate microcosms were averaged (Fig. 3). Results indicate that CO_2 production was not only linked to 2,4-D biodegradation, but also to mineralization of other organic substrates. Some of the highest 2,4-D biodegradation rates were in groundwaters with also the highest CO_2 production (i.e., 22-2). However, CO_2 production was higher than that expected from complete 2,4-D mineralization (0.006 mmol CO_2). For example, 22–2 which demonstrated to be the fastest 2,4-D degrading microcosm, produced 0.06 mmol CO_2 on day 34 (Fig. 3). The measured values thus correspond to CO_2 produced by DOC mineralization, either DOC from intrinsic groundwater or amended DOC. Additionally, CO_2 production continued even after complete depletion of 2,4-D was observed on day 42, although after day 50, the CO_2 production was lower. The reduction in CO_2 production can reflect less active microorganisms, possibly due to DOC or trace elements depletion. Hence, CO_2 measurements can be a general indication of microbial activity and to a certain extent, more activity can be related with faster 2,4-D biodegradation.

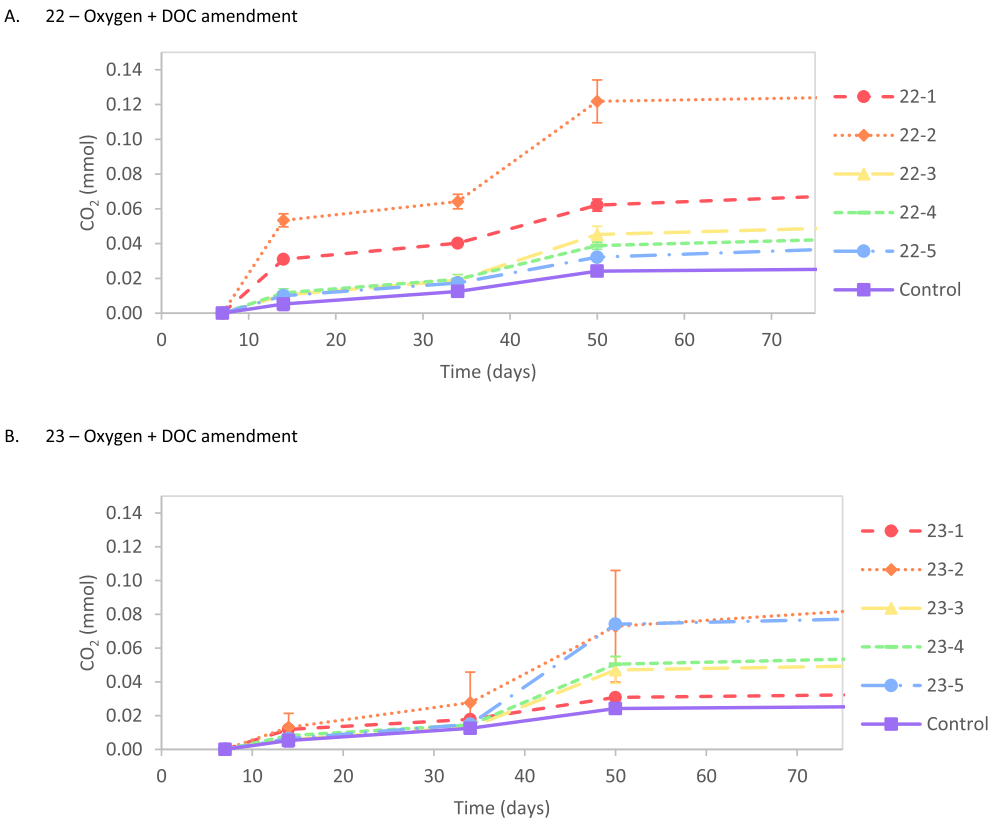

Fig. 3. CO_2 production from day 0–75. Triplicate groundwater samples from well 22 (A) and well 23 (B) at different depths amended with Oxygen + DOC.

3.3. *Implications for biological remediation technologies*

We successfully evaluated the MPs biodegradation capacity of groundwater from a drinking water abstraction aquifer. Amendment with oxygen + DOC supported 2,4-D biodegradation in groundwater from different locations and depth. In real field conditions, where not only water but also sediment is present, the impact of such amendment can be even larger. In our study, we only had access to groundwater samples. Thus we evaluated the biodegradation capacity of planktonic microorganisms. However, groundwater microorganisms are known to be present in lower abundance than biofilm forming microorganisms (Fillinger et al., 2019; Hug et al., 2015). We expect that microorganisms attached to sediments as biofilms also have biodegradation capacity and, due to their higher abundance, also could have a higher biodegradation activity upon addition of oxygen + DOC.

By the use of microcosm experiments, we found out that there is potential for the application of biostimulation as an in situ remediation technology. Field conditions can be different for instance in terms of temperature. We observed degradation in a matter of weeks in laboratory experiments at 20 °C, in groundwater the temperature is lower but also the time for treatment is longer than in laboratory. Thus, positive results in laboratory are hopeful for field application, but additional research on the effects of lower incubation temperatures should be conducted. Application of such technology should focus on treating the shallow part of the aquifer, where we observed higher biodegradation rates, likely due to higher microbial abundance or adaptation (Fig. 2). Moreover, our results also show that DOC contains microorganisms capable of MP biodegradation. Thus, addition of DOC extracted from natural sources could act then as both a biostimulant for intrinsic microorganisms, but also as bioaugmentation inoculum with active microorganisms. Although we did not observe BAM and MCPP biodegradation during 213 days of incubation, there is the possibility that in a longer period of time or with different amendments also more recalcitrant MPs can be degraded (Luo et al., 2019; Mierzejewska et al., 2019; Vandermaesen et al., 2016).

In-situ remediation technologies to remove iron from groundwater commonly aerate groundwater to precipitate iron-oxides. This technology enables the naturally present iron (Table 1) to be oxidized by aeration and to remove harmful contaminants such as metals or antibiotics from the aquifer (Du et al., 2020; Pi et al., 2017; Wang et al., 2019). This practice is an opportunity for DOC amendment to enhance the biodegradation capacity of field microorganisms. Essentially, after the water is being extracted, a DOC solution can be added to the extracted water while it is aerated. Afterwards, the aerated water amended with DOC can be deposited back in the wells.

The influence of DOC on the growth of aquifer microorganisms requires careful evaluation before full-scale application, since in a previous study, the biomass concentration increased after DOC addition (Luo et al., 2019). Higher DOC concentrations would be especially risky into the distribution network since it can cause microbial growth in the pipes, hampering drinking water production. Besides, since the DOC solutions can contain different microorganisms, the microbial communities of different DOC sources should be evaluated to ensure that no pathogenic bacteria is being added to groundwater aquifers. All in all, this study provides additional insights for further application of biological remediation technologies to mitigate low concentration pollution of pesticides and other MPs. Technologies that can be implemented in groundwater systems to secure safe drinking water production from clean aquifers.

4. Conclusion

We determined the natural biodegradation capacity of microorganisms in groundwater samples from different locations and various depths in a drinking water abstraction aquifer. We assessed biodegradation of BAM, MCPP, and 2,4-D by amending groundwater with nitrate + DOC,

oxygen + DOC, oxygen, and DOC. BAM and MCPP were not biodegraded in any well-sample at any amendment. Amendment with oxygen + DOC resulted in complete 2,4-D biodegradation within 42 days. Other amendments did not support 2,4-D biodegradation. Our results suggest that the rate of co-metabolic aerobic 2,4-D biodegradation depends on the microbial community, as we observed different degradation rates for different groundwater samples. DOC plays an important role in the biodegradation of MPs. Intrinsic DOC in groundwater did not support microbial activity or MPs biodegradation in the presence of oxygen, indicating that DOC quality selects for biodegradation activity. The present study demonstrates that oxygen + DOC was the best combination to support 2,4-D biodegradation. Further research is necessary to translate these findings into a technology to stimulate 2,4-D and other MPs biodegradation in situ in the field.

Credit author statement

Andrea Aldas-Vargas: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Thomas van der Vooren: Formal analysis, Investigation, Data curation, Huub H. M. Rijnaarts: Conceptualization, Writing – review & editing, Supervision, Nora B. Sutton: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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