

**Abstract: RELATING DISSOLVED ORGANIC MATTER AND BACTERIAL BIOMASS IN THE ENGLISH AQUIFER SYSTEMS**

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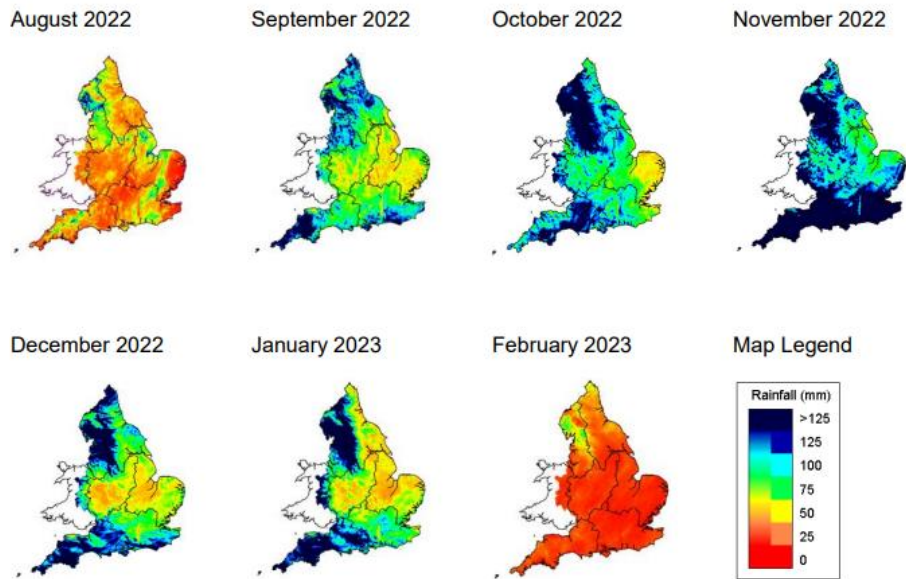
**Supplementary 1. Is the bacterial abundance affected by aquifer recharge?**

Groundwater is replenished with fresh input of nutrients and bacterial load from the surface by rainfall recharge (Baker et al., 2000; Griebler et al., 2010). The suspended bacterial density was found to shift with a time lag after a recharge event and then gradually return to their baseline over several weeks (Knobloch et al., 2021; Reiss et al., 2019; Zhou et al., 2012). To test if seasonal recharge affect bacterial density in English aquifers, we collected the pre-recharge season samples in late September to early October of 2022 and the post-recharge season samples in early February of 2023. To gather evidence of recharge process in the aquifer we looked at the following:

1. Rainfall report published by Environmental Agency between August 2022 and February 2023;
2. E. coli analysis of all samples.

The Environmental Agency's Water Situation report revealed unexpectedly dry recharge season in the study area during sampling. There was a lack of rainfall in the pre-recharge sampling period, i.e., August-September of 2022. Before the post-recharge sampling period, i.e., in December 2022-January 2023, much of the area covered by dual porosity aquifer received rainfall. In contrast, the areas covered by intergranular and karstic aquifer received only spatially patchy rainfall (Fig.S1). It is likely that recharge occurred throughout the dual porosity aquifer, and the observed drop in the median TCC in post-recharge season was the result of a dilution effect. The patchy post-recharge rainfall in the other two aquifers suggested recharge process was spatially patchy. Therefore TCC might have shifted in some places, but not in the others, making the resulting median TCC similar in both seasons.

E. coli analysis was performed by plate cultivation method using CHROMagar™ E.coli nutrient broth following standard product protocol. No E. coli colonies formed in any sample from all the aquifers and in both seasons. This indicated, none of the samples in any season represented a bacterial population disturbed by fresh surface water input (Sorensen et al., 2018).



**Supplementary Figure 1 (S1): Monthly rainfall across England during the sampling period revealed an unusually low rainfall. (Image copied from: [Water situation report for England February 2023 \(publishing.service.gov.uk\)](https://www.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/111111/water-situation-report-for-england-february-2023.pdf) )**

**Supplementary 2. Does land-use affect bacterial abundance?**

Land use in SPZ 1	Intergranular		Dual porosity	
	Pre-recharge	Post recharge	Pre-recharge	Post recharge
Forest cover	$\rho=0.22, p>0.1$	$\rho=0.09$	$\rho=-0.06$	$\rho=0.05$
Grassland	$\rho=-0.18, p>0.1$	$\rho=0.25, p>0.1$	$\rho=0.03$	$\rho=0.27, p>0.1$
Agricultural land	$\rho=-0.10, p>0.1$	$\rho=-0.13, p>0.1$	$\rho=0.11, p>0.1$	$\rho=0.06$
Urban area	$\rho=0.02$	$\rho=-0.24, p>0.1$	$\rho=0.22, p>0.1$	$\rho=-0.05$
Suburban area	$\rho=-0.05$	$\rho=0.05$	$\rho=0.18, p>0.1$	$\rho=-0.01$
Surface water	$\rho=0.16, p>0.1$	$\rho=0.18, p>0.1$	$\rho=0.42, p<0.05$	$\rho=0.27, p>0.1$

**Supplementary Table 1: Spearman correlations between land use categories and TCC. No karstic as n=8.**

An aquifer catchment's land-use pattern reportedly influences the groundwater bacterial community assemblage (Korbel et al., 2013; Sinreich et al., 2014; Stein et al., 2010). However, the land-use pattern did not seem to control the bacterial density (Fillinger et al., 2019).

To assess the effect of land-use patterns we calculated the coverage of different land-use categories from the UKCEH 2019 land cover map (Morton et al., 2020) in the source protection zone-1 of each pumping borehole (Agency, 2019) using the ArcGIS Pro zonal statistics tool. The analysed land-use categories were forest cover, grassland, agricultural land, urban and suburban areas, and surface water. No meaningful correlation between the proportional coverage of any land-use category and the bacterial TCC were observed (Supplementary Table 1).

## References:

- Agency, E. (2019). Manual for the production of Groundwater Source Protection Zones. In: Environment Agency Bristol.
- Baker, M. A., Valett, H. M., & Dahm, C. N. (2000). Organic carbon supply and metabolism in a shallow groundwater ecosystem. *Ecology*, *81*(11), 3133-3148.
- Fillinger, L., Hug, K., Trimbach, A. M., Wang, H., Kellermann, C., Meyer, A., Bendinger, B., & Griebler, C. (2019). The DA-(C) index: A practical approach towards the microbiological-ecological monitoring of groundwater ecosystems. *Water Research*, *163*, 114902.
- Griebler, C., Stein, H., Kellermann, C., Berkhoff, S., Brielmann, H., Schmidt, S., Selesi, D., Steube, C., Fuchs, A., & Hahn, H. J. (2010). Ecological assessment of groundwater ecosystems—vision or illusion? *Ecological Engineering*, *36*(9), 1174-1190.
- Knobloch, S., Klonowski, A. M., Tómasdóttir, S., Kristjánsson, B. R., Guðmundsson, S., & Marteinsson, V. P. (2021). Microbial intrusion and seasonal dynamics in the groundwater microbiome of a porous basaltic rock aquifer used as municipal water reservoir. *FEMS Microbiology Ecology*, *97*(3), fiab014.
- Korbel, K., Hancock, P., Serov, P., Lim, R., & Hose, G. (2013). Groundwater ecosystems vary with land use across a mixed agricultural landscape. *Journal of Environmental Quality*, *42*(2), 380-390.
- Morton, R. D., Marston, C. G., O'Neil, A. W., & Rowland, C. S. (2020). *Land Cover Map 2019 (land parcels, GB)* NERC Environmental Information Data Centre. <https://doi.org/10.5285/44c23778-4a73-4a8f-875f-89b23b91ecf8>
- Reiss, J., Perkins, D. M., Fussmann, K. E., Krause, S., Canhoto, C., Romeijn, P., & Robertson, A. L. (2019). Groundwater flooding: Ecosystem structure following an extreme recharge event. *Science of the Total Environment*, *652*, 1252-1260.
- Sinreich, M., Pronk, M., & Kozel, R. (2014). Microbiological monitoring and classification of karst springs. *Environmental Earth Sciences*, *71*, 563-572.
- Sorensen, J., Vivanco, A., Ascott, M., Gooddy, D., Lapworth, D., Read, D., Rushworth, C., Bucknall, J., Herbert, K., & Karapanos, I. (2018). Online fluorescence spectroscopy for the real-time evaluation of the microbial quality of drinking water. *Water Research*, *137*, 301-309.
- Stein, H., Kellermann, C., Schmidt, S. I., Brielmann, H., Steube, C., Berkhoff, S. E., Fuchs, A., Hahn, H. J., Thulin, B., & Griebler, C. (2010). The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater quality. *Journal of Environmental Monitoring*, *12*(1), 242-254.
- Zhou, Y., Kellermann, C., & Griebler, C. (2012). Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer. *FEMS Microbiology Ecology*, *81*(1), 230-242.

## Acknowledgement:

The rainfall maps are available at [Water situation report for England February 2023 \(publishing.service.gov.uk\)](https://www.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/91232/water-situation-report-for-england-february-2023.pdf)