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Abstract

Soil treatment units (STUs) receiving effluent from on-site wastewater treatment systems (OWTSs) rely on the gradual development of a microbial biomat/biozone at the infiltrative surface for optimal effluent distribution and pollutant attenuation. Here, we present the first direct measurement of gradual biomat development in the field in STU trenches receiving either primary (PE) or secondary treated effluent (SE) under identical environmental, hydrological and subsoil conditions. Two domestic OWTSs were constructed in Ireland and monitored over a period of >2 years using an automated, three-dimensional network of buried soil water content sensors tracking water flow and retention within the soil underneath the infiltrative surface. While trenches receiving PE expressed signs of biomat formation along the entire length of STU trenches, biomats in trenches receiving SE did not extend further than 10 m from the inlet at the end of the study. Fitting a decaying exponential growth model to the field data revealed, for the first time, that biomats in SE trenches will remain considerably shorter and not spread along the entire trench design length, even after 10 years of operation as compared to biomats in PE trenches (contrary to common design assumptions). This muted biomat growth in SE trenches has been demonstrated in the past to lead to localized hydraulic and pollutant overloading to the soil and has the potential to negatively affect the ability of the STU to attenuate pollutants effectively within the soil profile before the effluent reaches the groundwater.

Keywords	bioclogging; soil water content; wastewater treatment; hydraulic conductivity; septic system; vadose zone
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Cover Letter

Dear editor

Please find attached the research paper “***The influence of pre-treatment level on biomat development in soil treatment units***” for consideration for publication in the Journal of Contaminant Hydrology.

Soil treatment units (STUs) are prevalent discharge pathways in on-site wastewater treatment systems (OWTSs) and provide physical, chemical, and biological treatment for domestic effluent before it finally reaches the groundwater. Globally, a growing number of households are served by OWTSs, especially in regions currently under-served with basic needs services; in the EU and the US, an estimated 20% of the population currently uses on-site systems for wastewater treatment. To protect the underlying aquifer, STUs rely on the gradual development of a microbial biomat/biozone within the soil. This paper represents the first comparative field-scale study where the influence of pre-treatment (primary treated effluent vs. secondary treated effluent) on biomat development and water/effluent distribution within the STU was studied. Two full-scale treatment systems were constructed and monitored for >2.5 years using a three-dimensional network of embedded soil sensors tracing changes in volumetric water content within the STU as a proxy for biomat growth. Previous work has either been limited to lab-scale soil mesocosms or compared separate field-scale systems, i.e. systems that differed when it comes to effluent loading, weather, or soil conditions.

The results of this paper are in line with the expectations on biomat development from previous studies but is the first paper to present direct experimental evidence. The paper clearly enhances the understanding of environmental processes related to the natural remediation of polluted water sources in the unsaturated zone and, thus, addresses a human impact on the contamination of water resources at field-scale (i.e., the discharge of domestic effluent from on-site systems). The paper discusses control methods designed to eliminate or reduce these impacts (i.e., the potential need to revise current one-size-fits-all design guidelines for soil treatment units) and concludes that the chosen experimental approach can be replicated to gain further understanding of the temporal development of biomats in on-site treatment systems.

Hence, we consider this paper to be suitable for publication in the Journal of Contaminant Hydrology.

This original manuscript has not been previously published and is not being submitted to any other journal for consideration. In addition, for the preparation of this manuscript we have consulted the Instructions to Authors and confirm that this paper follows the Ethical Guidelines. All authors have read the manuscript and agreed to submit to the Journal of Contaminant Hydrology.

Kind regards,

A handwritten signature in blue ink, appearing to read 'Jan Knappe', is written over a light blue horizontal line.

Jan Knappe
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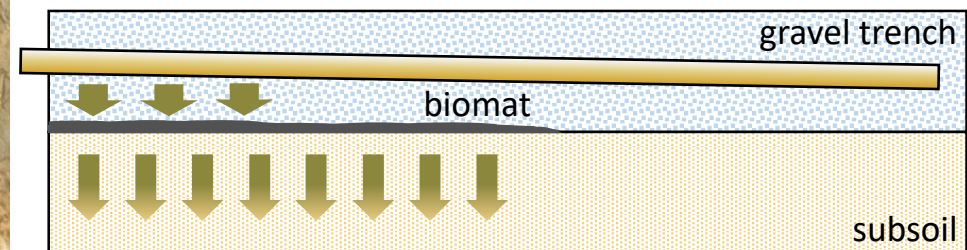
HIGHLIGHTS

- Automated soil sensor networks can be used to trace biomat development
- Biomats act as protective hydraulic barrier in the soil
- Trenches receiving septic tank effluent clogged within the first two years
- Biomat growth in trenches receiving secondary treated effluent was muted
- Incomplete biomat development can lead to hydraulic and pollutant overloading

Soil Treatment Unit (STU)

Primary effluent

Secondary effluent



The influence of pre-treatment on biomat development in soil treatment units

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ABSTRACT

Soil treatment units (STUs) receiving effluent from on-site wastewater treatment systems (OWTSs) rely on the gradual development of a microbial biomat/biozone at the infiltrative surface for optimal effluent distribution and pollutant attenuation. Here, we present the first direct measurement of gradual biomat development in the field in STU trenches receiving either primary (PE) or secondary treated effluent (SE) under identical environmental, hydrological and subsoil conditions. Two domestic OWTSs were constructed in Ireland and monitored over a period of >2 years using an automated, three-dimensional network of buried soil water content sensors tracking water flow and retention within the soil underneath the infiltrative surface. While trenches receiving PE expressed signs of biomat formation along the entire length of STU trenches, biomats in trenches receiving SE did not extend further than 10 m from the inlet at the end of the study. Fitting a decaying exponential growth model to the field data revealed, for the first time, that biomats in SE trenches will remain considerably

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shorter and not spread along the entire trench design length, even after 10 years of operation as compared to biomats in PE trenches (contrary to common design assumptions). This muted biomat growth in SE trenches has been demonstrated in the past to lead to localized hydraulic and pollutant overloading to the soil and has the potential to negatively affect the ability of the STU to attenuate pollutants effectively within the soil profile before the effluent reaches the groundwater.

KEYWORDS

bioclogging; soil water content; on-site wastewater treatment; hydraulic conductivity; septic system; vadose zone

ABBREVIATIONS

COD, chemical oxygen demand; EPS, extracellular polymeric substance; HER, hydrologically effective rainfall; LTAR, long-term acceptance rate; OWTS, on-site wastewater treatment system; PE, primary treated effluent; SD, standard deviation; SE, secondary treated effluent; SEE, standard error of the estimate; SMD, soil moisture deficit; STU, soil treatment unit; TN, total nitrogen; TOC, total organic carbon; VWC, volumetric water content

NOMENCLATURE

ET_0 Penman-Monteith reference evapotranspiration; ET_a actual evapotranspiration; K_{eff} effective hydraulic conductivity; K_{sat} saturated hydraulic conductivity; l_{bio} biomat length; l_{ss} steady-state biomat length; r_c linear biomat growth rate; t time; μ initial biomat growth rate; θ_r residual volumetric water content; θ_s saturated volumetric water content

1 INTRODUCTION

On-site wastewater treatment systems (OWTSs) are small scale, decentralized solutions for the treatment of domestic effluent and serve a considerable proportion of the population worldwide (Massoud et al., 2009). In both Europe and the United States, an estimated 20% of the population relies on OWTS (Eurostat, 2013; U.S. EPA, 2016). In Ireland, with its relatively

large share of dispersed settlements, approximately 489,000 OWTs are installed, serving a total of 38% of the population (CSO, 2017).

OWTs commonly consist of a septic tank followed by an optional secondary treatment unit and some form of soil treatment unit (STU) for final disposal (Cooper et al., 2016; Gill et al., 2009; McKinley and Siegrist, 2011; Siegrist et al., 2012). The primary treated effluent (PE), also called septic tank effluent (STE), still has high organic, nutrient, and pathogen content and, if not further treated, needs to be safely discharged to soil via an engineered STU as it still poses considerable risks to public and environmental health if discharged to surface water bodies (Amador and Loomis, 2018; EPA, 2009). Packaged, secondary treatment units which employ a variety of aerobic and anaerobic processes are sometimes added to the treatment train before the STU to further reduce the pollutant load. In Ireland, the inclusion of packaged systems producing secondary treated effluent (SE) prior to the STU depends *inter alia* on subsoil type, water table depth and the vulnerability of the underlying aquifer and is regulated by a Code of Practice (EPA, 2009).

STUs are engineered systems to facilitate the effective distribution and treatment of effluent in the subsoil (Amador and Loomis, 2018; Gill et al., 2009). STU design can vary from traditional soakaway systems and more commonly employed trench- or bed-based infiltration areas to pressurized drip emitters or layered sand filters buried in the ground (Amador and Loomis, 2018; Gill et al., 2015; Rowan, 2016; Siegrist, 2017). Studies on the long-term behavior of effluent infiltration in STUs showed that with time a microbial clogging zone, called the biomat or biozone, establishes in the upper few centimeters of the soil (Beal et al., 2006; McKinley and Siegrist, 2011, 2010; Rainwater et al., 2005; Rice, 1974; Siegrist and Boyle, 1987). While the presence of this clogging zone was historically often linked to hydraulic failure of STUs leading to insufficient effluent infiltration and potential ponding of effluent above ground (de Vries, 1972; McGauhey and Winneberger, 1964), the gradual formation of a biomat along the trench base is now understood to be crucial for facilitating effective effluent distribution over the entire design area and pollutant removal through adsorption, biodegradation and filtration

76 within the biomat itself and the unsaturated soil beneath (Amador and Loomis, 2018; Beal et
77 al., 2005; McKinley and Siegrist, 2011; Siegrist et al., 2012).

78 Previous research established links between the effective steady-state, long-term hydraulic
79 resistance and biomat thickness to subsoil characteristics, effluent organic and suspended
80 solids content and the entrapment of gas bubbles in the soil (McKinley and Siegrist, 2011, 2010;
81 Rice, 1974; Siegrist and Boyle, 1987; Ye et al., 2009). Reduced pollutant attenuation and
82 hydraulic overloading in the soil profile beneath STUs has previously been linked to muted long-
83 term biomat development in systems receiving SE as compared to PE (Gill et al., 2009).
84 However, the temporal development of biomats in STU trenches in the field, especially their
85 horizontal and vertical growth, has received only limited targeted research to date as the
86 influence of widely varying system-specific characteristics such as subsoil type, effluent loading,
87 effluent quality and weather make direct comparisons between STUs difficult to interpret. E.g.,
88 while studies on soil mesocosms showed that systems including secondary units before soil
89 treatment exceeded total nitrogen (TN) removal capabilities of a conventional system (Cooper
90 et al., 2015), field studies found that providing secondary treatment might limit biomat growth
91 to only a few meters along STU trenches, resulting in significantly reduced attenuation of TN in
92 the soil (Gill et al., 2009).

93 In this study, we present the results of the first direct assessment of biomat development in
94 full-scale STUs and its influence on effluent distribution, water retention and hydraulic
95 conductivity in trenches receiving PE and SE under the same environmental, hydrological and
96 subsoil conditions. The objectives of this study were to investigate the suitability of embedded
97 soil water content sensors for tracing biomat development, to quantify the impact of pre-
98 treatment on biomat growth and to assess the impact of a growing biomat on water retention
99 and changes in hydraulic conductivity in the soil.

100 **2 STUDY DESIGN AND METHODS**

101 **2.1 FIELD RESEARCH SITES**

Two OWTs were built and instrumented for this study serving fully occupied rural houses in Co. Limerick, Ireland according to the guidelines laid out in the Irish Code of Practice for Wastewater Treatment and Disposal Systems Serving Single Households (EPA, 2009). The first part of the treatment train for both systems consisted of a two-chamber prefabricated concrete septic tank (Aswasep Septic Tank NS4S, Molloy Precast, Ireland) with a capacity of 4760 L (Table 1). The septic tank effluent was split into two equal streams using a tipping bucket distribution box (Patel et al., 2008). One half of the effluent was directly delivered into two STU trenches as PE and the other half of the effluent underwent additional treatment before being delivered into two additional STU trenches as SE (Figure 1A). On Site A, the secondary treatment unit consisted of an intermittently-dosed media filter (Ecoflo Coco Filter, Premier Tech Aqua Ltd., Ireland). Site B was equipped with a continuous-flow two-stage rotating biological contactor (RBC) with secondary clarification chamber (Klargester BioDisc, Kingspan, UK). The STU trenches were 18 m long, 0.50 m wide and spaced 2.5 m apart. A perforated distribution pipe was laid in the trenches filled with washed gravel (20 to 30 mm diameter) at a slope of 1:200. A layer of geotextile (T1000, Terram, UK) separated the top of the trench from the overlaying backfilled soil and prevented the ingress of fine soil particles (Figure 1B).

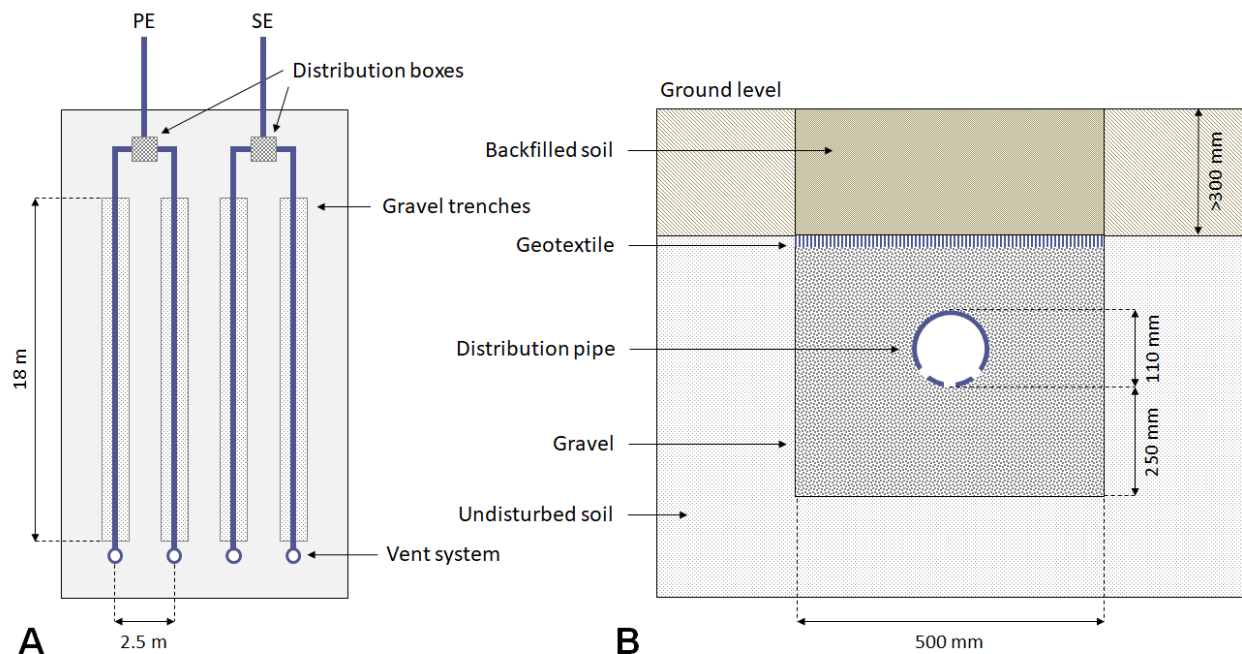


Figure 1. (A) Plan view of the STU. (B) Section of a STU gravel trench.

Site A and Site B were monitored for a total of 1080 and 860 days of operation, respectively. Subsoil testing revealed that Site A was located in a moderately draining sandy loam with saturated hydraulic conductivity $K_{sat} = 30.9 \text{ cm d}^{-1}$ (SD 3.5) and Site B was located on a slightly slower draining clay loam with $K_{sat} = 13.9 \text{ cm d}^{-1}$ (SD 5.4) as determined by a series of percolation tests ($n = 4$) performed on undisturbed subsoil using a constant-head permeameter (Aardvark, Soilmoisture, USA).

Table 1. Overview of site characteristics and installed system

Parameter	Site A	Site B
Location	Kilmallock, Co. Limerick	Crecora, Co. Limerick
Subsoil type	Sandy Loam	Clay Loam
Groundwater table ^(a)	1.6 m	>2.5 m
K_{sat}	30.9 cm d ⁻¹ (SD 3.5)	13.9 cm d ⁻¹ (SD 5.4)
Construction	September 2015	April 2016
Primary treatment	Septic tank	Septic tank
Secondary treatment	Cocopeat media filter	Rotating biological contactor
Flow regime	Pumped flow	Gravity flow
Number of occupants	5	4

^(a) At the time of site assessment

2.2 INSTRUMENTATION

Meteorological data was collected via automated weather stations (Campbell Scientific, UK) installed on each site measuring air temperature, relative humidity, atmospheric pressure, net radiation, wind speed and direction, and rainfall at hourly intervals. The hydraulic load to the OWTs was monitored using reed switches mounted in calibrated tipping bucket flow distribution boxes (Patel et al., 2008). Effluent samples were collected as grab samples during

site visits ($n = 30$ for Site A and $n = 28$ for Site B) from the distribution boxes and stored on ice for <6 h during transport before being analyzed in the Environmental Engineering Laboratory at Trinity College Dublin. The organic load of the PE and SE fed into STU trenches was determined as chemical oxygen demand (COD) using dichromate digestion cell test kits (Merck, Germany) and as total organic carbon (TOC) using a Shimadzu TOC-V analyzer (Shimadzu Scientific Instruments, USA). Nitrogen loading was measured as total nitrogen (TN) using a TNM-L module (Shimadzu Scientific Instruments, USA), and as nitrate-nitrogen ($\text{NO}_3\text{-N}$), nitrite-nitrogen ($\text{NO}_2\text{-N}$) and ammonium-nitrogen ($\text{NH}_4\text{-N}$) using a Konelab 20i chemistry analyzer (Thermo Scientific, Finland). To assess the spatial distribution of volumetric water content (VWC) and long-term changes of water retention within the STU, a network of 80 and 92 soil moisture sensors (EC5, Decagon Devices, USA) was installed during site construction on Site A and Site B, respectively, at depths of 5 cm, 10 cm and 15 cm below the infiltrative surface of STU trenches at various distances from the inlet (Figure S1). Control sensors were placed outside the STU in undisturbed soil at depths corresponding to STU sensors. Site-specific sensor calibrations were performed according to manufacturer recommendations using soil extracted from test holes prior to site construction.

2.3 BIOMAT HYDRAULIC CONDUCTIVITY

To quantify the influence of a growing biomat on the hydraulic properties of the soil, constant-head permeameter tests were conducted at the end of the study by using a constant-head permeameter (Aardvark, Soilmoisture, USA) inserted into cut lengths of drain pipes (100 mm diameter) placed directly onto the infiltrative surface at distances of 1 m, 8 m and 12 m from the inlet. The effective hydraulic conductivity (K_{eff}) of the infiltrative surface was then calculated using a generalized model describing water infiltration through the base of a lined test hole (Wu et al., 1999).

2.4 DATA ANALYSIS

Data from the weather stations was used to calculate Penman-Monteith reference evapotranspiration (ET_0), actual evapotranspiration (ET_a), hydrologically effective rainfall (HER) and soil moisture deficit (SMD) as daily aggregates (Allen et al., 1998; Limbrick, 2002;

Schulte et al., 2005). VWC readings were aggregated to daily means across pre-treatment type for every sensor position and normalized using site- and depth-specific residual and saturated VWC values, θ_r and θ_s , obtained from HYDRUS 1D simulations to account for the effects of localized heterogeneities in the soil on apparent sensor readings. HYDRUS simulation parameters are presented in the Supporting Information (Table S1) (Šimůnek et al., 2013). Sensor time series with oscillating behavior caused by air pockets introduced during sensor installation were flagged using k-means clustering on time series summary statistics (mean, inter quartile range, skewness, kurtosis) and discarded (2.5% and 1.1% of the data for Site A and Site B, respectively). Missing observations due to temporary sensor outages were gap filled by applying a smooth Bayesian time series fitting algorithm implemented with linear trend term, seasonality terms, and logistic growth term using θ_r and θ_s as lower and upper bound (17.4% and 4.8% of the data for Site A and Site B, respectively) (Taylor and Letham, 2018). All data analysis was performed using R, version 3.5.1 (R Core Team, 2018).

3 RESULTS AND DISCUSSION

3.1 EFFLUENT LOADINGS

Hydraulic and organic loading into the systems was monitored throughout the study. Site A and Site B experienced mean daily effluent flows into the STU of 269.8 L d⁻¹ (SD 329.1) and 500.1 L d⁻¹ (SD 200.8), respectively. The high variability in flows observed at Site A is a result of the pumped flow regime as compared to the gravity flow employed on Site B. Observed mean organic concentrations when expressed as COD in the PE of 606 mg L⁻¹ (SD 241) and 1005 mg L⁻¹ (SD 193) were lowered to 221 mg L⁻¹ (SD 116) and 67 mg L⁻¹ (SD 50) in the SE at Site A and Site B, respectively. Similarly, TOC concentrations in the PE were lowered from 163 mg L⁻¹ (SD 83) and 304 mg L⁻¹ (SD 55) to 64 mg L⁻¹ (SD 42) and 27 mg L⁻¹ (SD 16) in the SE at Site A and Site B, respectively. While the RBC installed at Site B removed a mean 93% of the organic load when expressed as COD, the media filter at Site A only achieved a 64% COD reduction. This is most likely a direct result of the drastically lower hydraulic retention time (HRT) of the media filter as compared to the RBC (order of minutes vs. order of hours). TN concentrations at Site A were

168 mg L⁻¹ (SD 69) with an average of 37.4% as NH₄-N and 1.6% as NO₃-N and 116 mg L⁻¹ (SD 45) with an average of 20.9% as NH₄-N and 38.0% as NO₃-N for PE and SE respectively. At Site B, TN concentrations were 245 mg L⁻¹ (SD 23) with an average of 34.5% as NH₄-N and 0.3% as NO₃-N and 24 mg L⁻¹ (SD 29) with an average of 11.1% as NH₄-N and 56.5% as NO₃-N for PE and SE respectively. NO₂-N concentrations remained <1% of TN throughout the study for both sites and effluent types.

3.2 METEOROLOGICAL CONDITIONS

During the study period, the sites received a mean annual rainfall of 928.6 mm and 972.4 mm at mean temperatures of 10.0 °C and 10.4 °C with record lows of -7.2 °C and -7.4 °C and highs of 30.8 °C and 30.3 °C for Site A and Site B, respectively. Detailed meteorological time series of monthly rainfall, actual evapotranspiration and temperature data are presented in the Supporting Information (Figure S2). A seasonal flooding event following a period of extended heavy rainfall in winter 2015/16 led to temporary hydraulic failure of the system at Site A for a period of approximately 2 months after which normal functioning resumed. Summer 2018 was marked by an extended period of drought persisting for approximately two months leading to severe soil drying (Met Éireann, 2018).

3.3 CONTROL SENSORS

As expected, the shallower control soil moisture sensors showed distinct responses to seasonal and rainfall patterns, whereas deeper sensors were less affected by environmental factors exhibiting a lagged response and generally higher VWC (Figure S3). The drought in summer 2018 was clearly marked by dramatic drying of the natural soil. The VWC at 10 cm below ground decreased by approximately 0.03 cm³ cm⁻³ and 0.04 cm³ cm⁻³ within weeks after the onset of dry conditions reaching the overall lowest VWC values recorded during the study, i.e. 0.105 cm³ cm⁻³ and 0.176 cm³ cm⁻³ at Site A and Site B respectively.

3.4 BIOMAT GROWTH

3.4.1 WATER RETENTION IN THE BIOMAT

Biomat development at the infiltrative surface of STU trenches is marked by gradual physical and biological pore clogging and increased water retention in the biozone as a direct result of the development of an extracellular polymeric substance (EPS) matrix in the void space (Adessi et al., 2018; Lennon and Lehmkuhl, 2016; Roberson and Firestone, 1992; Volk et al., 2016). Depending on substrate availability and mass transfer processes, biomat growth will cause increased resistance to water flow (Rosenzweig et al., 2014) and, thus, contribute to the eventual distribution of effluent along the entire length of STU trenches (Beach et al., 2005; McKinley and Siegrist, 2011; Siegrist and Boyle, 1987; Winstanley and Fowler, 2013). As soil microorganisms are regularly challenged by changing moisture conditions potentially reducing substrate diffusion, restricting motility and experiencing desiccation stress in the soil environment, a mature biomat may serve as a protective barrier to ensure efficient soil treatment and variably saturated condition in the underlying soil (Geza et al., 2013; McKinley and Siegrist, 2011; Potts, 1994; Roberson and Firestone, 1992).

The vertical extent of the biomat was limited to the upper few centimeters of the soil. Long-term changes in water retention within the soil underlying the infiltrative surface suggest that the effect of improved water retention generally diminished both with horizontal distance from the inlet and depth below the trench base (Figure 2). At Site A, soil sensors expressed a mean $0.015 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.007) and $0.009 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.008) increase in water content before the onset of a severe drought in summer 2018 in trenches receiving PE and SE, respectively (Figure 2). The effect was most pronounced at the 5 cm sensor position with mean water content increases of $0.014 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.008) and diminished to $0.009 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.006) and $0.003 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.002) at depths of 10 cm and 15 cm, respectively. Similarly, in Site B trenches receiving PE expressed a higher overall mean water content increase of $0.012 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.008) as compared to trenches receiving SE with $0.009 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.007). Highest water retention was, again, found in the upper layer with $0.015 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.006), but was considerably lower at depth with $0.004 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.005) at 10 cm (Figure 2). The lowest sensors located at 15 cm depth expressed a slight overall decrease in water content with $-0.001 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.003). This indicates that, independent of the level of pre-treatment, once a biomat has formed it can act as a water retaining, protective barrier as previously described in

laboratory-scale experiments and by numerical simulations (Beach et al., 2005; Beach and McCray, 2003).

During dry conditions, the biomat formed a protective barrier effectively increasing the resilience of soil microbes towards desiccation stress by retaining additional moisture within the EPS matrix. This preserved the hydraulic connectivity of microbial communities and access to aqueous and gaseous substrates (Guo et al., 2018; Hobley et al., 2013; Roberson and Firestone, 1992; Tamaru et al., 2005). From 19 May to 12 August 2018 an extensive drought prevailed over Ireland and no HER was recorded on either site. During this period, the hydraulic load to STUs was solely comprised of the effluent fed into the trenches and changes in water retention within the upper layer of soil below the infiltrative surface were indicative of the presence or absence of a hydraulically protective barrier, i.e. the biomat. Changes of VWC from the onset of the dry conditions until the end of this study are presented in Figure 3. Two general patterns were observed: (i) Site A expressed a larger decrease across both trench types as compared to Site B with changes of $-0.028 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.015) and $-0.006 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.011), respectively, which is in line with the respective saturated hydraulic conductivities of each site (Table 1); and (ii) overall losses in water content were higher in trenches receiving SE as compared to PE with changes of $-0.023 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.018) and $-0.010 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.012) respectively, indicating that the differences of substrate availability for biomat growth in trenches receiving PE and SE led to the formation of a biomat that was able to withstand drying stress more readily in PE trenches as compared SE trenches. Except for trenches receiving PE in Site B (which was partially ponded during excavation at the end of the study), all trenches expressed the highest mean loss of water content during the dry period within the upper 5 cm with $-0.022 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.009), $-0.039 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.016), $-0.001 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.002) and $-0.012 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.012) as compared to lower depths with $-0.018 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.009), $-0.023 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.016), $-0.000 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.005) and $-0.013 \text{ cm}^3 \text{ cm}^{-3}$ (SD 0.014) for Site A PE, Site A SE, Site B PE and Site B SE, respectively, albeit with a generally decreasing magnitude with increasing distance from the inlet. In regions where the biomat was present and no to low desiccation occurred within the soil profile, the presence of biofilm EPS resulted in a hydraulic decoupling of the biomat-affected soil and the dry conditions (Figure 3 and S3). This response

to environmental stress may effectively act to protect the embedded bacteria and limit the exchange of water content with the surrounding porous soil while also leading to a potential increase in microbial community diversity and resilience to other environmental stressors (Guo et al., 2018; Or et al., 2007).

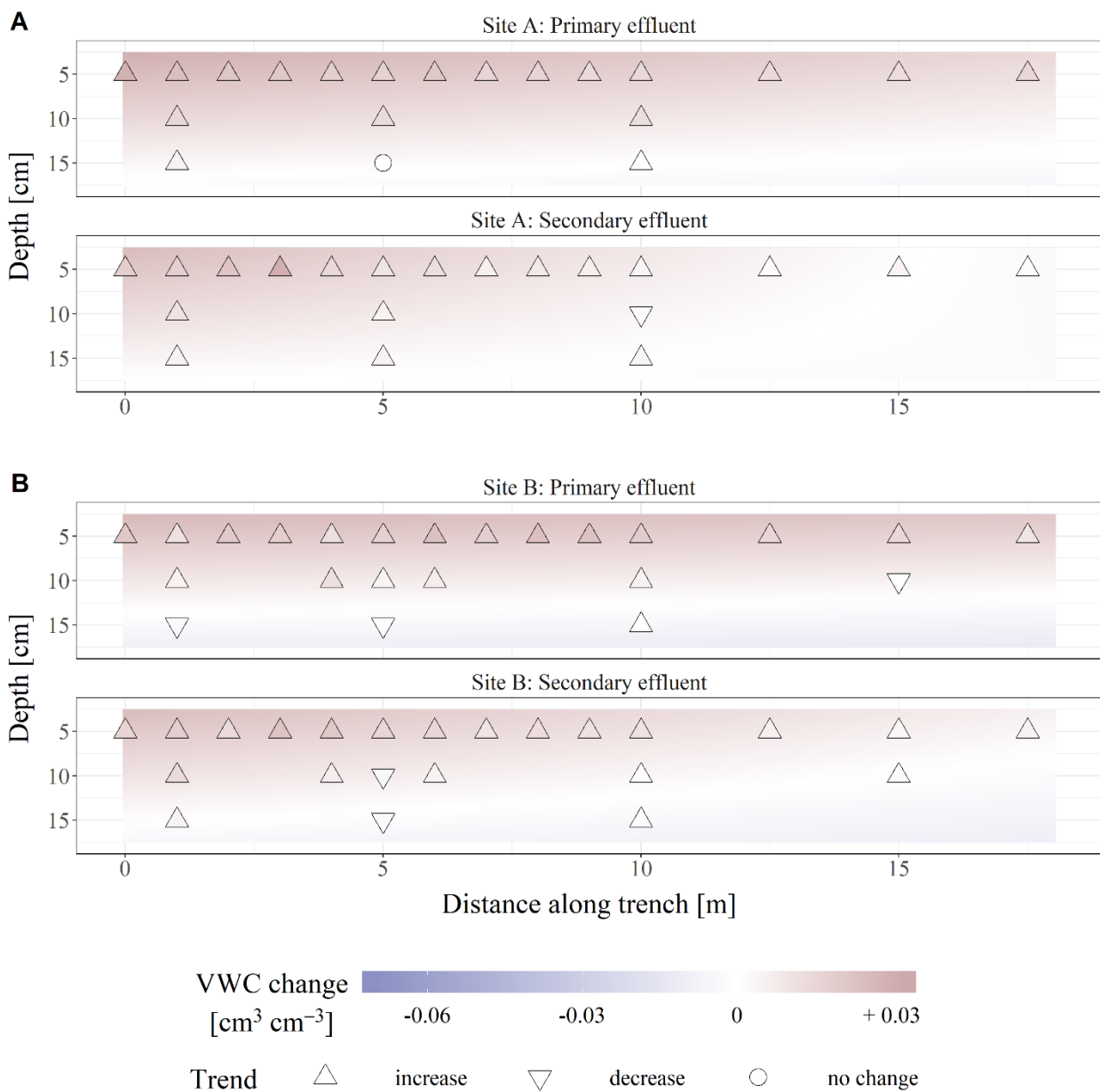


Figure 2. Spatial variation of changes in volumetric water content (VWC) from the start of operation until the onset of the drought in summer 2018 for the soil beneath the infiltrative surface in trenches receiving primary and secondary effluent in Site A (panel A) and Site B (panel B). Changes are represented by upward and downward facing pyramids for increases and decreases in VWC, respectively. Positions where no significant changes as compared to initial VWC conditions were observed are marked by circles. The fill represents the magnitude of the

observed change. Values are spatially interpolated between observation points using a two-dimensional linear model for improved visualization.

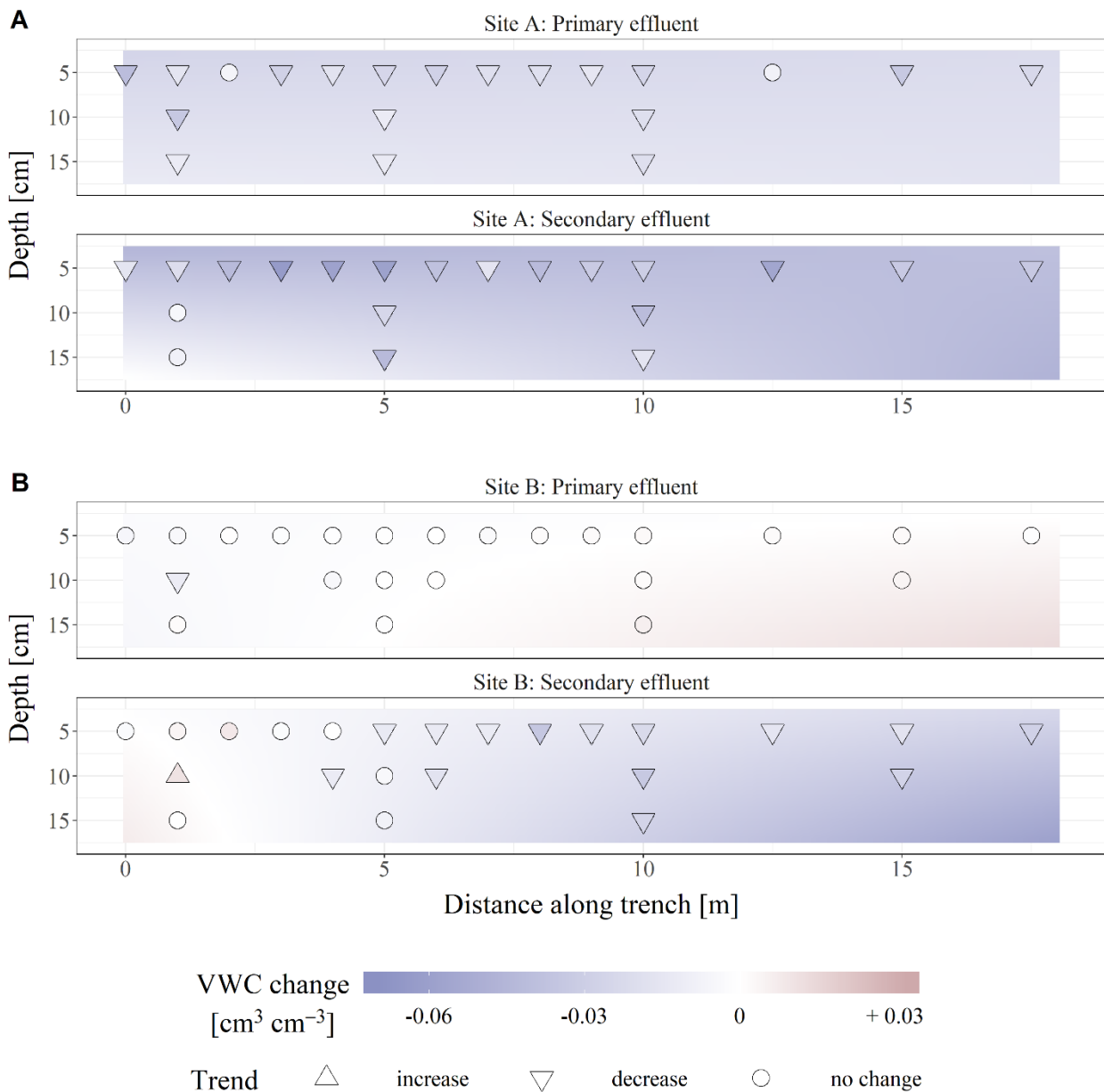


Figure 3. Spatial variation of changes in volumetric water content (VWC) during the drought in summer 2018 for the soil beneath the infiltrative surface in trenches receiving primary and secondary effluent in Site A (panel A) and Site B (panel B). Changes are represented by upward and downward facing pyramids for increases and decreases in VWC, respectively. Positions where no significant changes as compared to initial VWC conditions were observed are marked by circles. The fill represents the magnitude of the observed change. Values are spatially interpolated between observation points using a two-dimensional linear model for improved visualization.

3.4.2 BIOMAT GROWTH RATES

Horizontal biomat growth rates were strongly influenced by the level of pre-treatment. Using data obtained by the shallow soil moisture sensors installed at a depth of 5 cm, biomat establishment at a given sensor location was determined as the time to reach a mean $0.025 \text{ cm}^3 \text{ cm}^{-3}$ increase in VWC over initial baseline level (i. e. mean VWC during the first week of operation) for a continuous period of at least 30 days. STU sensor data was corrected for relative changes observed at control sensors installed at the same depth to eliminate the influence of environmental parameters on changes in observed VWC changes. The temporal threshold of 30-days was chosen to further reduce the detection of false positives due to heavy rainfall events and short-term weather-related water content changes. The $0.025 \text{ cm}^3 \text{ cm}^{-3}$ cut-off level was chosen after considering both the range of previously observed and modeled changes in VWC over time where the increase in water content and a concomitant decrease in the infiltration rate was attributed to clogging of soil pores (Beal et al., 2008; Cook et al., 1994; Volk et al., 2016).

In both sites, clogging extended to all positions along the trenches receiving PE until 15 m from the inlet, although at a slower rate in Site B. While clogging occurred within the first six months of operation for positions closer than 5 m, it took approximately 10 and 13 months for the biomat to reach a length of 15 m at Site A and Site B, respectively (Figure 4). In trenches receiving SE, biomat growth did not extend to more than 7.5 m and 10 m after approximately three years of operation at Site A and Site B, respectively (Figure 4). This indicates that the low organic content in the SE (as compared to PE) may be limiting biomat growth, as both hydraulic loading and subsoil conditions were identical between trenches receiving SE and PE at both sites.

Two basic mathematical models were fitted to the resulting data. A linear model predicted the length of the biomat (l_{bio}) as a function of time (t) and a linear biomat growth rate (r_c) as

$$l_{bio} = r_c t \quad (\text{Eq. 1})$$

assuming that biomat growth was not limited by substrate availability, i.e. carbon content in the effluent. In this model biomat development progresses at a steady rate over time until the entire trench base is fully covered with biomat, i. e. complete clogging occurred. Biomat growth in the linear model is conceptually unlimited but, in reality, limited by the length of the trench. Once complete clogging is reached, ponding may occur if the long-term acceptance rate (LTAR) is below the hydraulic loading rate (Beal et al., 2006; Winstanley and Fowler, 2013). Where possible, a second, non-linear, model was fitted to the data to obtain an estimate for the biomat length (l_{bio}) using a steady-state biomat length (l_{ss}) by including an exponential decay as

$$l_{bio} = l_{ss}[1 - e^{-\mu t}] \quad (\text{Eq. 2})$$

which is scaled by an initial biomat growth rate μ to account for the slowing of biomat growth with time t if substrate availability is a limiting factor for biomat growth.

While the non-linear model performed well on data obtained from trenches receiving SE, it did not converge on data from trenches receiving PE within physically meaningful boundaries. The linear model predicted biomat growth rates of $4.06 \times 10^{-2} \text{ cm d}^{-1}$ (SEE 0.50) and $3.13 \times 10^{-2} \text{ cm d}^{-1}$ (SEE 0.24) for trenches receiving PE and $1.17 \times 10^{-2} \text{ cm d}^{-1}$ (SEE 0.08) and $1.46 \times 10^{-2} \text{ cm d}^{-1}$ (SEE 0.14) for trenches receiving SE in Site A and Site B, respectively (Table 2). With these biomat growth rates, full trench clogging in trenches receiving PE should have occurred after 13 to 16 months of operation at Site A and 18 to 21 months of operation at Site B. Both estimates were well within the overall observation period for the sites. However, neither STU experienced clogging during the study at positions more than 15 m from the inlet, potentially indicating that either biomat growth did not occur here or that, if a biomat had formed, its impact on water retention was not yet as pronounced as closer to the inlet. In trenches receiving SE, biomat growth was significantly muted (by factor >2) compared to trenches receiving PE. With biomat growth rates predicted by the linear model, full trench clogging should have occurred after 48 to 55 months of operation at Site A and 33 to 48 months of operation at Site B; well beyond the observation period for both sites. However, as previous

studies have shown, it is likely that biomats in trenches receiving SE will not grow to the full extent of STU trenches. (Amador and Loomis, 2018; Gill et al., 2009). For Site A, the growth limiting non-linear model predicted a final steady-state biomat length of 8.9 m to 13.9 m (95 % confidence intervals) after approximately 6 to 10 years of operation (Table 2). For Site B, the final biomat length in trenches receiving SE estimated with the non-linear model was 10.7 m to 17.1 m (95 % confidence intervals) after approximately 5 to 7 years of operation (Table 2). Both biomat length estimates are slightly higher than the observed values of 7.5 m and 10 m for Site A and Site B, respectively, but also represent a longer time period than the <3 years of observation in this study.

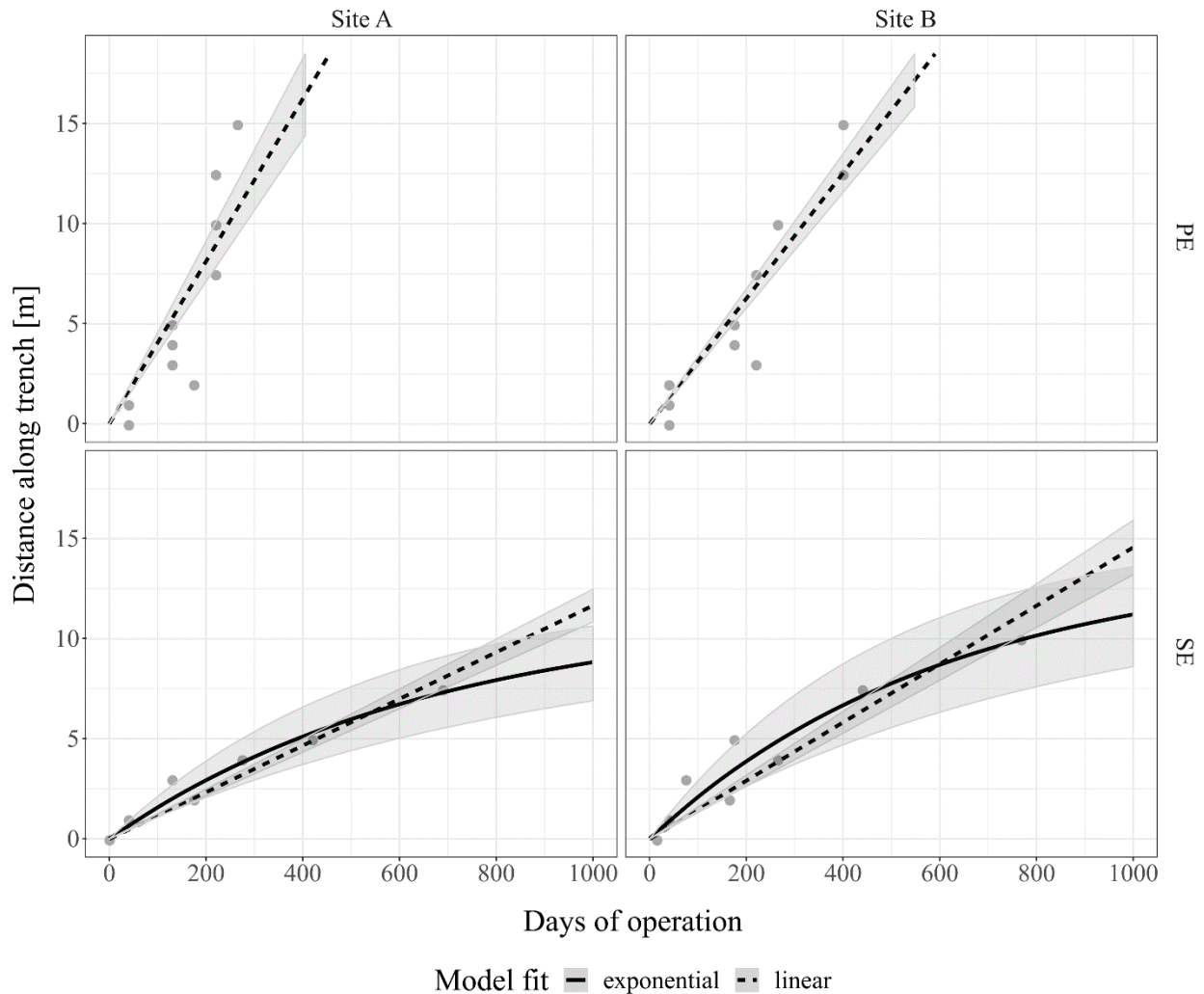


Figure 4. Biommat development for Site A and Site B in trenches receiving primary (PE) and secondary (SE) effluent. Onset of clogging (solid gray circles) was determined as the earliest point in time to reach a consistent 2.5% elevated mean volumetric water content above base level at 5 cm depth for a duration of at least 30 d. Dashed and

solid lines represent linear and exponential fits with 95% confidence intervals (gray shaded areas), respectively. Fit results are presented in Table 2.

Table 2. Results of linear and exponential model fit for horizontal biomat development.

Site	Effluent ^(a)	Model ^(b)	Coefficients ^(c)
Site A	PE	linear	$r_c = 4.06 \times 10^{-2} \text{ cm d}^{-1}$ (SEE 0.50)
	SE	linear	$r_c = 1.17 \times 10^{-2} \text{ cm d}^{-1}$ (SEE 0.08)
		exponential	$l_{ss} = 11.41 \text{ m}$ (SEE 1.27)
			$\mu = 1.63 \times 10^{-3} \text{ d}^{-1}$ (SEE 0.42)
Site B	PE	linear	$r_c = 3.13 \times 10^{-2} \text{ cm d}^{-1}$ (SEE 0.24)
	SE	linear	$r_c = 1.46 \times 10^{-2} \text{ cm d}^{-1}$ (SEE 0.14)
		exponential	$l_{ss} = 13.95 \text{ m}$ (SEE 1.65)
			$\mu = 1.49 \times 10^{-3} \text{ d}^{-1}$ (SEE 0.34)

^(a) PE: primary treated effluent; SE: secondary treated effluent

^(b) linear model fit: $l_{bio} = r_c t$; exponential model fit: $l_{bio} = l_{ss}[1 - e^{-\mu t}]$

^(c) expressed as estimate \pm standard error of the estimate (SEE)

In summary, both sites expressed notable water retention above base and background levels in the upper soil layer beneath the trench base progressing from the inlet with biomat growth rates depending on pre-treatment. While biomat growth in trenches receiving PE appeared not to be limited by substrate availability and expressed no slowing down as biomat growth progressed within the first 15 m (with the result that unit area hydraulic loading rates decreased), biomat growth in trenches receiving SE followed a non-linear trend with retardation resulting in an eventual steady-state biomat length which was significantly shorter than the 18 m trenches. While biomat growth in trenches receiving SE was faster and the steady state biomat length was longer in Site B as compared to Site A, the opposite was observed in trenches receiving PE. Mathematical studies of biomat development in STU

trenches revealed that growth rates and biomat lengths express a higher sensitivity to microbial growth and decay rates as compared to soil conductivity and effluent substrate concentration (Winstanley and Fowler, 2013). However, the *in-situ* determination of biomat microbial growth and decay rates in the field remains experimentally challenging. Long-term biomat lengths as determined by the non-linear model, on the other hand, suggest that a muted spread of the biomat is both a function of effluent concentration and soil conductivity, as previously reported in field trials in Ireland (Gill et al., 2009).

3.4.3 EFFECTIVE HYDRAULIC CONDUCTIVITY OF THE BIOMAT

Biomat growth as a result of effluent application has been shown to drastically reduce the effective hydraulic conductivity of the soil, K_{eff} . While laboratory-scale studies primarily conducted on sand columns fed with effluent of various strength generally found conductivity reduction in the range of one to four orders of magnitude as compared to unaffected soil, (Beal et al., 2006; de Vries, 1972; Magesan et al., 2000; McGauhey and Winneberger, 1964; Rice, 1974; Rodgers et al., 2004; Volk et al., 2016) few studies have been conducted to date directly measuring biomat K_{eff} in the field: estimates range from 17.3 cm d⁻¹ on a clay soil fed with high sodium SE and 3.1 cm d⁻¹ for a sandy loam fed with artificial wastewater and a clay loam fed with PE, to 0.92 cm d⁻¹ for biomats on a loam soil fed with PEPE (Beal et al., 2008; Bouma, 1975; Gonçalves et al., 2007; Rainwater et al., 2005; Siegrist and Boyle, 1987). In this study, changes in apparent soil conductivity were strongly influenced by the level of pre-treatment and distance from the inlet (i.e. by the presence of a microbial biomat). Results of the permeameter tests measured directly on the base of the trenches after 860 and 1080 days of operation in Site A and Site B, respectively are presented in Table S2 and compared to the K_{sat} values obtained from the undisturbed soil for each site. In Site A, conductivity reductions from an initial 30.9 cm d⁻¹ in the natural soil to 9.7 and 12.1 cm d⁻¹ at distances of 8 m and 12 m from the inlet, respectively, measured directly on the trench base were found in trenches receiving PE. This reduction further indicated the drastic effects of pore clogging due to the presence of a microbial biomat at these positions on soil physical parameters. During excavations at the end of the study, distinct color changes to dark gray in the soil profile were indicative of microbial

activity and reduced conditions at these positions (Figure S4). Trenches receiving SE, unexpectedly, expressed an even greater conductivity reduction at the 1 m position (to 3.2 cm d⁻¹) but showed no significant reduction at the 8 m position, indicating no substantial biofilm formation at this position. Trench excavations at Site B revealed ponded conditions (approximately 10 cm head) in trenches receiving PE along the entire trench which is indicative of the presence of a mature biomat reducing the effective infiltration rate below the hydraulic loading rate of the STU. There was clear visual indication of anaerobic conditions and microbial growth on the surface of gravel submerged in effluent. K_{sat} was effectively reduced to approximately one third of the initial 13.9 cm d⁻¹ in the undisturbed soil to 3.1 cm d⁻¹ to 4.0 cm d⁻¹ along the trench base at the end of the study. SE trenches at Site B did not show signs of ponding and the conductivity reduction was limited to 12.5 cm d⁻¹ and 11.3 cm d⁻¹ at positions located 1 m and 8 m along the trench, respectively. Despite increased water content at these locations, this minimal reduction in infiltrative capacity might indicate that microbial growth affected smaller soil pores more than larger ones at this stage as suggested in previous studies.^{55,56} The dry conditions at the time of excavation during the summer might also have contributed to the observed limited K_{sat} reduction as compared to the entirely ponded trenches receiving PE by potentially reducing overall biofilm thickness within the soil matrix due to desiccation stress, which could be a focus of future studies.

4 CONCLUSIONS

- This study presents the first direct assessment of gradual biomat development and its influence on effluent distribution in STU trenches receiving PE and SE under the same environmental, hydrological and subsoil conditions.
- The results show that embedded, automated soil sensor networks can be deployed to trace long-term biomat development in the field by using changes in VWC as a proxy for gradual soil clogging. In the future, improvements in sensor technology might allow for higher spatial resolution monitoring as measurement volumes of soil sensors become smaller, thus allowing to detect biomat effects within the upper few cm beneath the infiltrative surface more accurately.

- Increased water retention in the biomat due to the formation of an EPS matrix in soil pores aided to reduce the effects of environmental stress, such as desiccation during extended dry periods, and increased the resilience of soil microbes to extreme weather events that will become more frequent in the future as the effects of anthropogenic climate change intensify.
- The long-term biomat length was strongly influenced by the level of pre-treatment with trenches receiving effluent with lower organic concentrations developing significantly shorter biomats, which has severe implications for the overall effectiveness of effluent distribution and subsequent pollutant attenuation within STUs if not properly considered while designing and planning OWTSSs.
- Further research should be directed towards studying biogeochemical transformation pathways in and the microbial composition of developing and mature biomats to gain improved understanding of biomat functioning in STUs.

AUTHOR CONTRIBUTIONS

LWG and ACF conceived the study and secured funding. JK and LWG planned the experiments. JK, CS and LWG performed the experiments and analyzed data. JK and LWG wrote the manuscript. All authors interpreted the results and reviewed the manuscript.

COMPETING INTERESTS

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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SUPPORTING INFORMATION

The Supporting Information for this manuscript includes:

- Figure S1: Positions of soil sensors used in this study
- Figure S2: Meteorological data
- Figure S3: Control soil sensor time series
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- Table S1: Simulation parameters for HYDRUS
- Table S2: Results of constant-head permeameter tests

REFERENCES

- Adessi, A., Cruz de Carvalho, R., De Philippis, R., Branquinho, C., Marques da Silva, J., 2018. Microbial extracellular polymeric substances improve water retention in dryland biological soil crusts. *Soil Biology and Biochemistry* 116, 67–69. doi:10.1016/j.soilbio.2017.10.002
- Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration: Guidelines for computing crop water requirements. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
- Amador, J.A., Loomis, G.W., 2018. Soil-based wastewater treatment, 1st ed. Soil Science Society of America, Inc., Madison, WI, USA.
- Beach, D.N.H., McCray, J.E., 2003. Numerical modeling of unsaturated flow in wastewater soil absorption systems. *Groundwater Monit. R.* 23, 64–72. doi:10.1111/j.1745-6592.2003.tb00672.x
- Beach, D.N.H., McCray, J.E., Lowe, K.S., Siegrist, R.L., 2005. Temporal changes in hydraulic conductivity of sand porous media biofilters during wastewater infiltration due to biomat formation. *J Hydrol (Amst)* 311, 230–243. doi:10.1016/j.jhydrol.2005.01.024
- Beal, C.D., Gardner, E.A., Kirchhof, G., Menzies, N.W., 2006. Long-term flow rates and biomat zone hydrology in soil columns receiving septic tank effluent. *Water Res.* 40, 2327–2338. doi:10.1016/j.watres.2006.04.018
- Beal, C.D., Gardner, E.A., Menzies, N.W., 2005. Process, performance, and pollution potential: A

481 review of septic tank - soil absorption systems. *Soil Res.* 43, 781. doi:10.1071/SR05018
 482 Beal, C.D., Rassam, D.W., Gardner, E.A., Kirchhof, G., Menzies, N.W., 2008. Influence of
 483 hydraulic loading and effluent flux on surface surcharging in soil absorption systems. *J.*
 484 *Hydrol. Eng.* 13, 681–692. doi:10.1061/(ASCE)1084-0699(2008)13:8(681)
 485 Bouma, J., 1975. Unsaturated Flow During Soil Treatment of Septic Tank Effluent. *Journal of the*
 486 *Environmental Engineering Division.*
 487 Cook, F.J., Kelliher, F.M., McMahon, S.D., 1994. Changes in Infiltration and Drainage during
 488 Wastewater Irrigation of a Highly Permeable Soil. *Journal of Environment Quality* 23,
 489 476. doi:10.2134/jeq1994.00472425002300030010x
 490 Cooper, J.A., Loomis, G.W., Amador, J.A., 2016. Hell and high water: diminished septic system
 491 performance in coastal regions due to climate change. *PLoS ONE* 11, e0162104.
 492 doi:10.1371/journal.pone.0162104
 493 Cooper, J.A., Loomis, G.W., Kalen, D.V., Amador, J.A., 2015. Evaluation of water quality
 494 functions of conventional and advanced soil-based onsite wastewater treatment
 495 systems. *J. Environ. Qual.* 44, 953–962. doi:10.2134/jeq2014.06.0277
 496 CSO, 2017. Census 2016 Summary Results- Part 1. Technical report, Central Statistics Office.
 497 Central Statistics Office, Dublin, Ireland.
 498 de Vries, J., 1972. Soil filtration of waste water effluent and the mechanism of pore clogging.
 499 *Journal of the Water Pollution Control Federation* 44, 565–573.
 500 EPA, 2009. Code of Practice: Wastewater Treatment and Disposal Systems Serving Single
 501 Households (p.e. < 10). Environmental Protection Agency, Wexford, Ireland.
 502 Eurostat, 2013. Eurostat database. URL <http://ec.europa.eu/eurostat/data/database> (accessed
 503 5.22.19).
 504 Geza, M., Lowe, K.S., Huntzinger, D.N., McCray, J.E., 2013. New Conceptual Model for Soil
 505 Treatment Units: Formation of Multiple Hydraulic Zones during Unsaturated
 506 Wastewater Infiltration. *J. Environ. Qual.* 42, 1196–1204. doi:10.2134/jeq2012.0441
 507 Gill, L.W., Dubber, D., O’Flaherty, V., Keegan, M., Kilroy, K., Curneen, S., Misstear, B., Johnston,
 508 P., Pilla, F., McCarthy, T., Qazi, N., Smyth, D., 2015. Assessment of disposal options for
 509 treated wastewater from single houses in low permeability subsoil, STRIVE Report

510 Series161. Environmental Protection Agency, Wexford, Ireland.

511 Gill, L.W., O’Luanaigh, N., Johnston, P.M., Misstear, B.D.R., O’Suilleabhain, C., 2009. Nutrient
512 loading on subsoils from on-site wastewater effluent, comparing septic tank and
513 secondary treatment systems. *Water Res.* 43, 2739–2749.
514 doi:10.1016/j.watres.2009.03.024

515 Gonçalves, R.A.B., Folegatti, M.V., Gloaguen, T.V., Libardi, P.L., Montes, C.R., Lucas, Y., Dias,
516 C.T.S., Melfi, A.J., 2007. Hydraulic conductivity of a soil irrigated with treated sewage
517 effluent. *Geoderma* 139, 241–248. doi:10.1016/j.geoderma.2007.01.021

518 Guo, Y.-S., Furrer, J.M., Kadilak, A.L., Hinestroza, H.F., Gage, D.J., Cho, Y.K., Shor, L.M., 2018.
519 Bacterial extracellular polymeric substances amplify water content variability at the
520 pore scale. *Front. Environ. Sci.* 6. doi:10.3389/fenvs.2018.00093

521 Hobley, L., Ostrowski, A., Rao, F.V., Bromley, K.M., Porter, M., Prescott, A.R., MacPhee, C.E., van
522 Aalten, D.M.F., Stanley-Wall, N.R., 2013. BslA is a self-assembling bacterial hydrophobin
523 that coats the *Bacillus subtilis* biofilm. *Proc Natl Acad Sci USA* 110, 13600–13605.
524 doi:10.1073/pnas.1306390110

525 Lennon, J.T., Lehmkuhl, B.K., 2016. A trait-based approach to bacterial biofilms in soil. *Environ.*
526 *Microbiol.* 18, 2732–2742. doi:10.1111/1462-2920.13331

527 Limbrick, K.J., 2002. Estimating daily recharge to the Chalk aquifer of southern England – a
528 simple methodology. *Hydrol. Earth Syst. Sci.* 6, 485–496. doi:10.5194/hess-6-485-2002

529 Magesan, G.N., Williamson, J.C., Yeates, G.W., Lloyd-Jones, A.R., 2000. Wastewater C:N ratio
530 effects on soil hydraulic conductivity and potential mechanisms for recovery. *Bioresour.*
531 *Technol.* 71, 21–27. doi:10.1016/S0960-8524(99)00054-1

532 Massoud, M.A., Tarhini, A., Nasr, J.A., 2009. Decentralized approaches to wastewater treatment
533 and management: applicability in developing countries. *J. Environ. Manage.* 90, 652–
534 659. doi:10.1016/j.jenvman.2008.07.001

535 McGauhey, P.H., Winneberger, J.H.T., 1964. Studies of the failure of septic tank percolation
536 systems. *Journal (Water Pollution Control Federation)* 36, 593–606.

537 McKinley, J.W., Siegrist, R.L., 2010. Accumulation of Organic Matter Components in Soil under
538 Conditions Imposed by Wastewater Infiltration. *Soil Science Society of America Journal*

539 74, 1690. doi:10.2136/sssaj2009.0395
 540 McKinley, J.W., Siegrist, R.L., 2011. Soil clogging genesis in soil treatment units used for onsite
 541 wastewater reclamation: A review. *Crit. Rev. Environ. Sci. Technol.* 41, 2186–2209.
 542 doi:10.1080/10643389.2010.497445
 543 Met Éireann, 2018. 2018, A summer of Heat Waves and Droughts. Met Éireann.
 544 Mostafa, M., Van Geel, P.J., 2007. Conceptual models and simulations for biological clogging in
 545 unsaturated soils. *Vadose Zone Journal* 6, 175. doi:10.2136/vzj2006.0033
 546 Mostafa, M., Van Geel, P.J., 2012. Validation of a relative permeability model for bioclogging in
 547 unsaturated soils. *Vadose Zone Journal* 11, 0. doi:10.2136/vzj2011.0044
 548 Or, D., Phutane, S., Dechesne, A., 2007. Extracellular Polymeric Substances Affecting Pore-Scale
 549 Hydrologic Conditions for Bacterial Activity in Unsaturated Soils. *Vadose Zone Journal* 6,
 550 298. doi:10.2136/vzj2006.0080
 551 Patel, T., O’Luanaigh, N., Gill, L.W., 2008. A Comparison of Gravity Distribution Devices Used in
 552 On-Site Domestic Wastewater Treatment Systems. *Water Air Soil Pollut.* 191, 55–69.
 553 doi:10.1007/s11270-007-9606-7
 554 Potts, M., 1994. Desiccation tolerance of prokaryotes. *Microbiol. Rev.* 58, 755–805.
 555 Rainwater, K., Jackson, A., Ingram, W., Lee, C.Y., Thompson, D., Mollhagen, T., Ramsey, H.,
 556 Urban, L., 2005. Field demonstration of the combined effects of absorption and
 557 evapotranspiration on septic system drainfield capacity. *Water Environ. Res.* 77, 150–
 558 161.
 559 Rice, R.C., 1974. Soil Clogging during Infiltration of Secondary Effluent. *Journal of Water*
 560 *Pollution Control Federation* 46, 708–716.
 561 Roberson, E.B., Firestone, M.K., 1992. Relationship between Desiccation and Exopolysaccharide
 562 Production in a Soil *Pseudomonas* sp. *Appl. Environ. Microbiol.* 58, 1284–1291.
 563 Rodgers, M., Mulqueen, J., Healy, M.G., 2004. Surface clogging in an intermittent stratified sand
 564 filter. *Soil Science Society of America Journal* 68, 1827. doi:10.2136/sssaj2004.1827
 565 Rosenzweig, R., Furman, A., Dosoretz, C., Shavit, U., 2014. Modeling biofilm dynamics and
 566 hydraulic properties in variably saturated soils using a channel network model. *Water*
 567 *Resour. Res.* 50, 5678–5697. doi:10.1002/2013WR015211

568 Rowan, M., 2016. Evaluation of drip irrigation emitters distributing primary and secondary
 569 wastewater effluents. *Irrigat. Drainage Sys. Eng.* 2. doi:10.4172/2168-9768.1000111
 570 R Core Team, 2018. R: A language and environment for statistical computing. Foundation for
 571 Statistical Computing, Vienna, Austria.
 572 Schulte, R.P., Diamond, J., Finkle, K., Holden, N.M., Brereton, A.J., 2005. Predicting the soil
 573 moisture conditions of Irish grasslands. *Irish Journal of Agricultural and Food Research*
 574 45, 95–110.
 575 Siegrist, R.L., 2017. *Decentralized Water Reclamation Engineering*, 1st ed. Springer, Basel,
 576 Switzerland.
 577 Siegrist, R.L., Boyle, W.C., 1987. Wastewater-induced soil clogging development. *J. Environ. Eng.*
 578 113, 550–566. doi:10.1061/(ASCE)0733-9372(1987)113:3(550)
 579 Siegrist, R.L., Lowe, K.S., Geza, M., McCray, J.E., 2012. Soil treatment units used for effluent
 580 infiltration and purification within onsite wastewater systems: Science and technology
 581 highlights, in: *Proceedings of the International Symposium on Domestic Waste Water*
 582 *Treatment and Disposal Systems*. Presented at the International Symposium on
 583 *Domestic Waste Water Treatment and Disposal Systems*, Dublin, Ireland, pp. 53–67.
 584 Šimůnek, J., Šejna, M., Saito, H., Sakai, M., van Genuchten, Mt., 2013. The Hydrus-1D Software
 585 Package for Simulating the Movement of Water, Heat, and Multiple Solutes in Variably
 586 Saturated Media, Version 4.17, HYDRUS Software Series 3. Department of
 587 Environmental Sciences, University of California Riverside, Riverside, California, USA.
 588 Tamaru, Y., Takani, Y., Yoshida, T., Sakamoto, T., 2005. Crucial role of extracellular
 589 polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium
 590 *Nostoc commune*. *Appl. Environ. Microbiol.* 71, 7327–7333.
 591 doi:10.1128/AEM.71.11.7327-7333.2005
 592 Taylor, S.J., Letham, B., 2018. Forecasting at Scale. *The American Statistician* 72, 37–45.
 593 doi:10.1080/00031305.2017.1380080
 594 U.S. EPA, 2016. *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2014*. U.S.
 595 Environmental Protection Agency, Washington DC, USA.
 596 Volk, E., Iden, S.C., Furman, A., Durner, W., Rosenzweig, R., 2016. Biofilm effect on soil hydraulic

597 properties: Experimental investigation using soil-grown real biofilm. *Water Resour. Res.*
 598 52, 5813–5828. doi:10.1002/2016WR018866
 599 Winstanley, H.F., Fowler, A.C., 2013. Biomat development in soil treatment units for on-site
 600 wastewater treatment. *Bull. Math. Biol.* 75, 1985–2001. doi:10.1007/s11538-013-9881-y
 601 Wu, L., Pan, L., Mitchell, J., Sanden, B., 1999. Measuring saturated hydraulic conductivity using
 602 a generalized solution for single-ring infiltrometers. *Soil Science Society of America*
 603 *Journal* 63, 788. doi:10.2136/sssaj1999.634788x
 604 Ye, S., Sleep, B.E., Chien, C., 2009. The impact of methanogenesis on flow and transport in
 605 coarse sand. *J. Contam. Hydrol.* 103, 48–57. doi:10.1016/j.jconhyd.2008.09.004
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Supporting Information

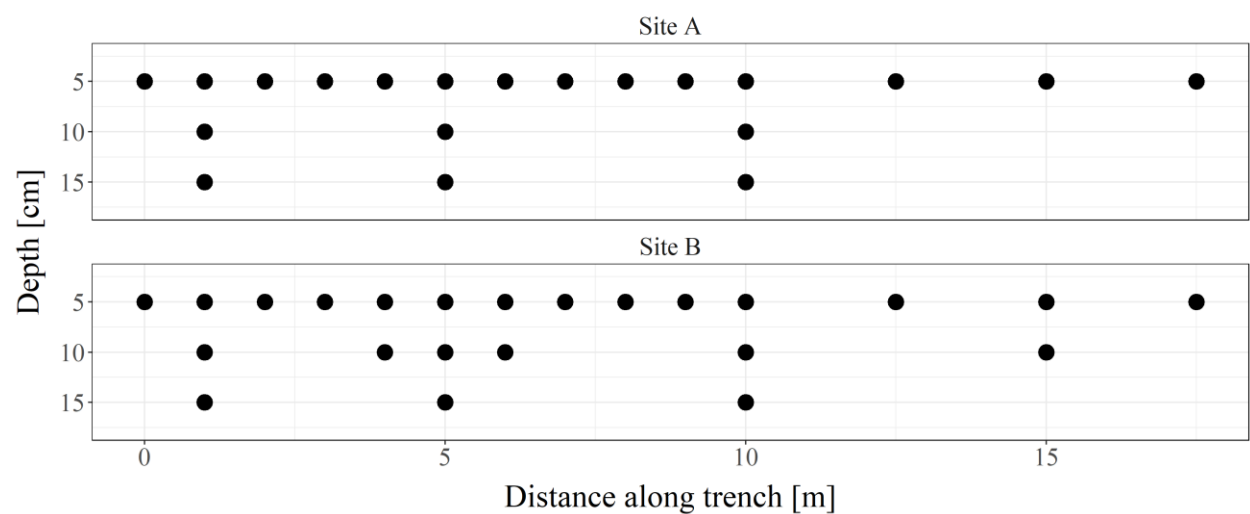
The influence of pre-treatment level on biomat development in soil treatment units

Jan Knappe, Celia Somlai, Andrew C. Fowler, Laurence W. Gill

The Supporting Information (7 pages) for this manuscript includes

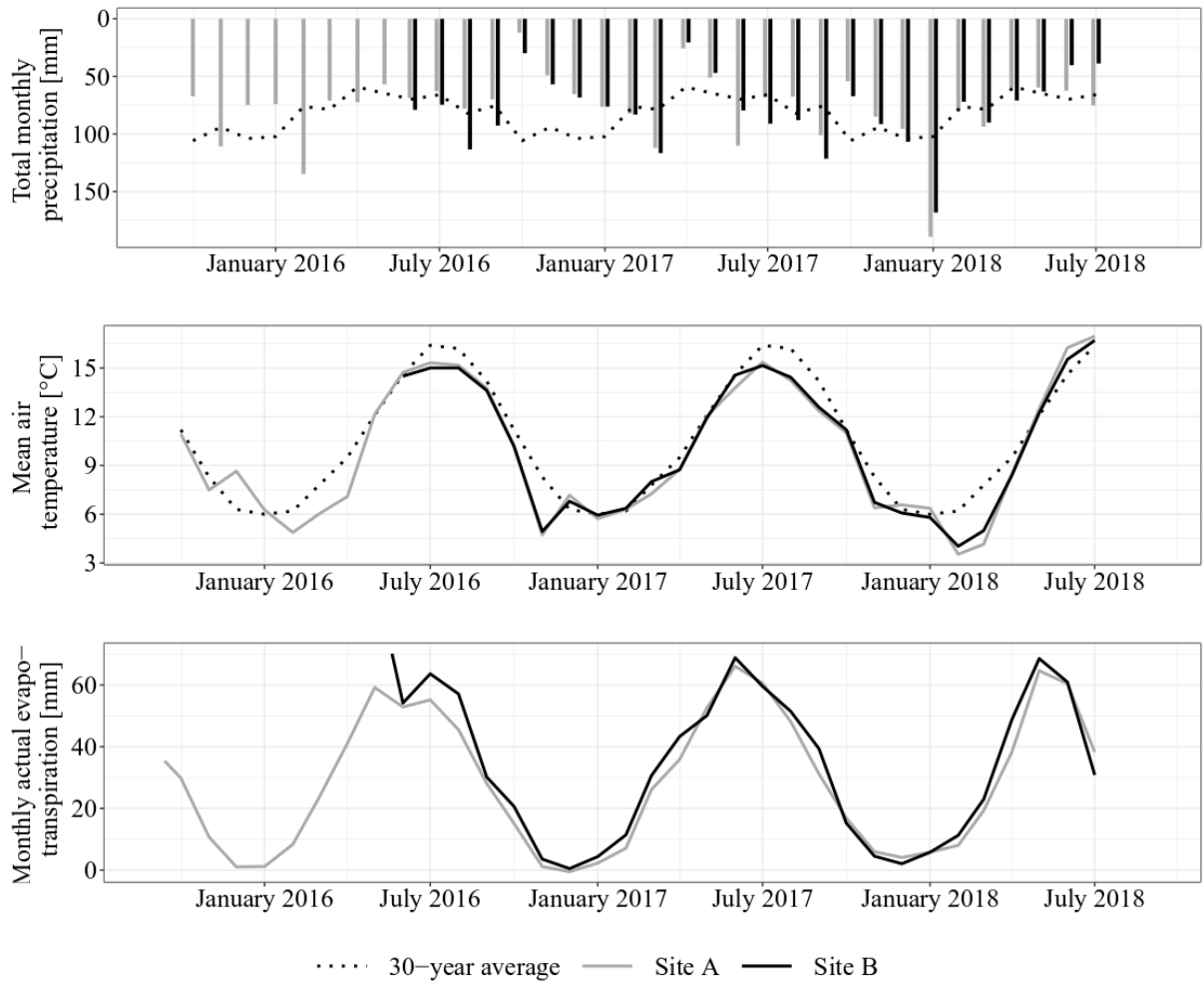
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618 **FIGURE S1**



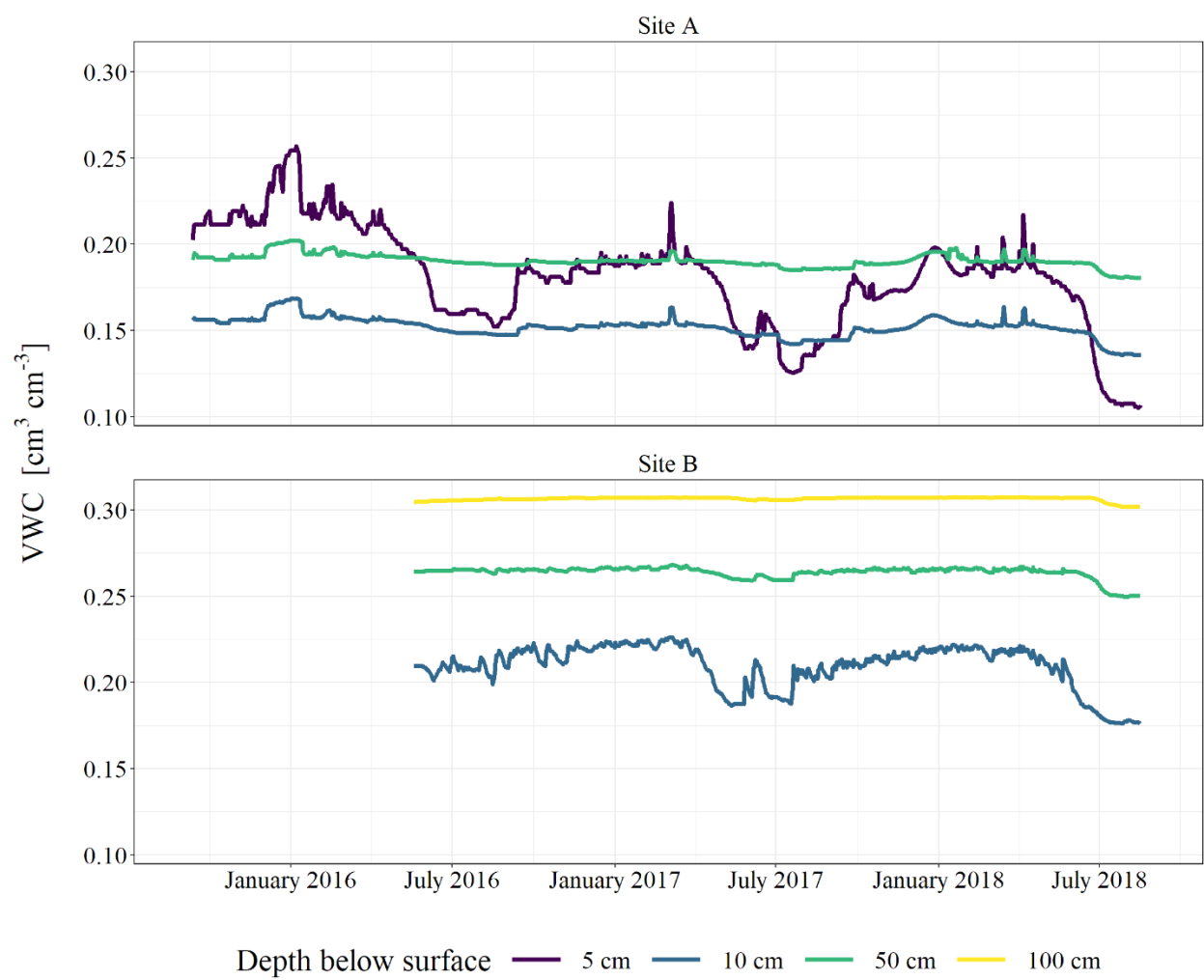
619 Figure S1. Positions of soil sensors underneath STU trenches at Site A and Site B. Each STU consisted of four
620 identical trenches. A total of 80 and 92 sensors were installed in Site A (upper panel) and Site B (lower panel),
621 respectively.

622 **FIGURE S2**



623 Figure S2. Monthly mean temperature, total reference evapotranspiration and total precipitation during the study for
624 Site A (gray line) and Site B (black line). Long-term (30-year) monthly averages for temperature and total
625 precipitation are shown as dotted lines for comparison (data from Shannon Airport, Co. Limerick, obtained from
626 Met Éireann).

627 **FIGURE S3**



628 Figure S3. Control soil sensor responses measuring volumetric water content (VWC) at various depths below the
629 soil surface for Site A (upper panel) and Site B (lower panel).

630 **FIGURE S4**



631 Figure S4. Example of soil cores taken from the trench base at the end of the study (shown here, core from Site A,
632 trench receiving primary effluent). Changes in soil coloration within the upper 5 cm indicate anaerobic conditions
633 within the biomat zone.

TABLE S1

Table S1. HYDRUS 1D simulations of a 1.5 m deep soil profile were performed using site specific parameters as given in this table with atmospheric boundary conditions on the top and free drainage conditions at the bottom. Continuous wetting was simulated by applying the site-specific mean daily rainfall and continuous drying conditions were simulated by applying the site-specific mean daily reference evapotranspiration to the atmospheric boundary. Simulations were run until a steady state was reached, upon which simulated water content profiles were used to inform the sensor signal normalization algorithm.

Site	θ_r	θ_s	α	n	K_s	l
Site A	0.0646	0.3769	0.0097 cm ⁻¹	1.222	0.0097 cm min ⁻¹	-1
Site B	0.0441	0.0384	0.0215 cm ⁻¹	1.4456	0.0215 cm min ⁻¹	-1

TABLE S2

Table S2. Results of constant-head permeameter tests for determining the field saturated hydraulic conductivity K_{fs} of the undisturbed subsoil and the effective saturated hydraulic conductivity K_{eff} at the base of percolation trenches receiving primary (PE) and secondary effluent (SE) after 1080 and 860 days of operation for Site A and Site B, respectively.

Site	K_{fs}	Effluent type	K_{eff}
Site A	30.9 cm d ⁻¹	PE	10.9 cm d ⁻¹
		SE	3.2 cm d ⁻¹
Site B	13.9 cm d ⁻¹	PE	3.6 cm d ⁻¹
		SE	11.9 cm d ⁻¹

Declaration of interests

☐ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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