ESSENTIAL TITLE PAGE INFORMATION

WOODCHIP BIOREACTORS PROVIDE SUSTAINED DENITRIFICATION OF BRINE FROM GROUNDWATER DESALINATION PLANTS

Carolina Díaz-García*, Juan J. Martínez-Sánchez*, Bryan M. Maxwell, José Antonio Franco, José Álvarez-Rogel

Present address: Departamento de Ingeniería Agronómica, E.T.S. de Ingeniería Agronómica, Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 48, Cartagena, 30203, Murcia, Spain.

* Corresponding author: Carolina Díaz-García carolina.diaz@upct.es

* Corresponding author: Juan J. Martínez-Sánchez: juan.martinez@upct.es

Bryan M. Maxwell: bmmaxwel@ncsu.edu

José Antonio Franco: josea.franco@upct.es

José Álvarez-Rogel: jose.alvarez@upct.es

WOODCHIP BIOREACTORS PROVIDE SUSTAINED DENITRIFICATION OF BRINE FROM GROUNDWATER DESALINATION PLANTS

Carolina Díaz-García*, Juan J. Martínez-Sánchez*, Bryan M. Maxwell, José Antonio Franco, José Álvarez-Rogel

Present address: Departamento de Ingeniería Agronómica, E.T.S. de Ingeniería Agronómica, Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 48, Cartagena, 30203, Murcia, Spain.

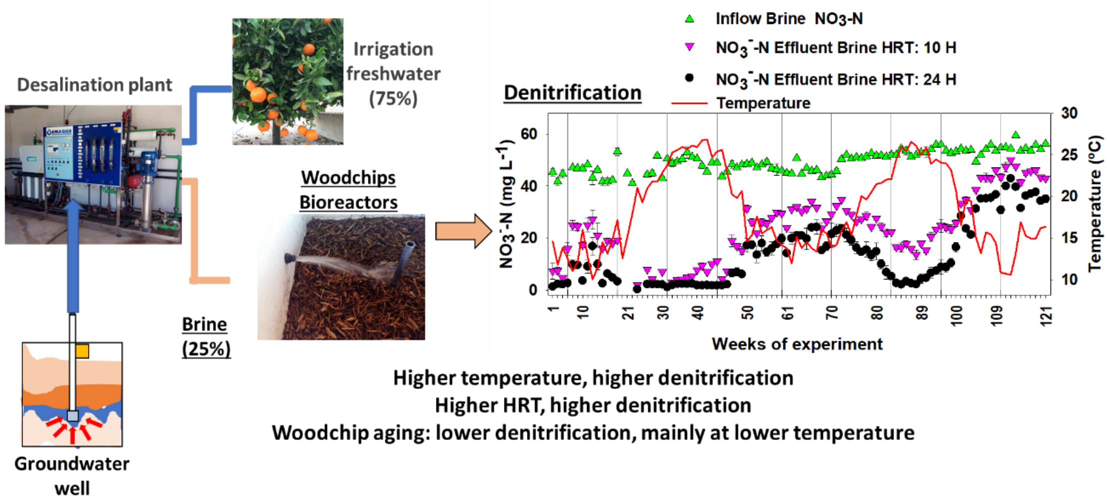
* Corresponding author: Carolina Díaz-García carolina.diaz@upct.es

* Corresponding author: Juan J. Martínez-Sánchez: juan.martinez@upct.es

Bryan M. Maxwell: bmmaxwel@ncsu.edu

José Antonio Franco: josea.franco@upct.es

José Álvarez-Rogel: jose.alvarez@upct.es



ABSTRACT

Woodchip bioreactors are widely known as a best management practice to reduce excess nitrate loads that are discharged with agricultural leachates. The aim of this study was to evaluate the performance of citrus woodchip bioreactors for denitrification of brine (electrical conductivity ≈ 17 mS cm^{-1}) from groundwater desalination plants with high nitrate content (NO_3^--N ≈ 48 mg L^{-1}) in the Campo de Cartagena agricultural watershed, one of the main providers of horticultural products in Europe. The performance was evaluated relative to seasonal changes in temperature, dissolved organic carbon (DOC) provided by woodchips, hydraulic residence time (HRT) and woodchip aging. Bioreactors (capacity 1 m^3) operated for 2.5 years (121 weeks) in batch mode (24 h HRT) with three batches per week. Denitrification efficiency was modulated by DOC concentration, temperature, hydraulic residence time and the drying-rewetting cycles. High salinity of brine did not prevent nitrate removal from occurring. The high DOC availability (> 25 mg C L^{-1}) during the first ≈48 weeks resulted in high nitrate removal rate (> 75 %) and nitrate removal efficiency (until ≈ 25 g N m^{-3} d^{-1}) regardless of temperature. Moreover, the high DOC contents in the effluents during this period may present environmental drawbacks. Denitrification was still high after 2.5 years (reaching ≈9.3 g N m^{-3} d^{-1} in week 121), but dependence on warm temperature became more apparent with woodchips aging from week ≈49 onwards. Nitrate removal efficiency was highest on the first weekly batch, immediately after woodchips had been unsaturated for four days. It was attributable to a flush of DOC produced by aerobic microbial metabolism during drying that stimulated denitrification following re-saturation. Hence, alternance of drying-rewetting cycles is an operation practice that increase bioreactors nitrate removal performance.

Keywords

Brine management; Nitrate removal; Batch bioreactors; Denitrification efficiency

1. Introduction

Demand of irrigation water for intensive agriculture has led to increased use of groundwater, particularly in Mediterranean semiarid areas with scarcity of surface water resources (IPCC, 2014). However, the quality of aquifers is many times compromised by high salinity, a problem that is expected to be aggravated according to predictions of the Intergovernmental Panel on Climate Change (Hoegh-Guldberg et al., 2018). Additionally, in intensive agricultural watersheds, groundwater resources are often polluted by nitrate (NO_3^-) due to fertilizer use for crop production (FAO, 2017). Nitrate contamination of surface and groundwater resources has become a major problem worldwide since it can lead to eutrophication, algae blooms, and fish kills in water bodies (Howarth, 2008; Lewis et al., 2011).

When withdrawals are made from aquifers with saline groundwater, it is often necessary to desalinate in order to meet the demands of activities such as tourism and irrigated agriculture (Palomar and Losada, 2010). The desalination process generates reject brine, which is a highly saline waste that can typically be discharged to the sea (Ministerio de Agricultura Alimentación y Medio Ambiente, 2013), or recovered for other uses such as frost control on roads (Lysbakken, 2013) hydrotherapy or environmental applications (Donnelly, 2014), irrigation of forage shrub and crops, or fish farming (Sánchez et al., 2015). However, additional brine management steps are required when groundwater contains other pollutants such as nitrate (NO_3^-), particularly when its concentration exceeds permissible legal release boundaries for discharges (Wisniewski et al., 2002; Beliavski et al., 2010; Bosko et al., 2014).

Previous studied have found high NO_3^- loads in reject brine from desalination plants, and different technologies have been applied to remove, including sequencing batch reactors with methanol as carbon substrate for denitrifier microorganisms (Xu et al., 2020), upflow sludge blanket reactors (Beliavski et al., 2010), membrane bioreactor (Wisniewski et al., 2002), fluidized bed absorber reactors (Ersever et al., 2007), electrodialysis (Bosko et al., 2014), and others. Although these technologies are capable of high NO_3^- reduction rates in brine, they are not well-suited for the agricultural application in local farms because they can be expensive and technically complex to manage. Denitrifying bioreactors are a potential alternative for this application since they can provide low-cost nitrate removal (Christianson et al., 2009), are easy to install, and require low maintenance (Schipper et al., 2010; Christianson and Helmers, 2011).

Denitrifying bioreactors consist of trenches or containers filled with a carbonaceous material (typically an organic waste or plant residue) through which the nitrate-enriched water is passes. The carbonaceous material provides organic carbon as an electron donor for anaerobic microorganisms to complete denitrification under suboxic/anoxic conditions (i.e., the transformation of NO_3^- into gaseous forms of nitrogen such as N_2O or N_2) (Schipper et al., 2010). A high organic carbon content is necessary since the lack of nutrients in brine hinder microbial proliferation, which may limit the application of biological technologies even when halophilic microorganisms are inoculated in reactors (Abou-Elela et al., 2010). Furthermore, widespread use of these bioreactors could increase the value of certain organic agricultural wastes if they can be used as carbon media in bioreactors (Díaz-García et al., 2020). Other advantages of woodchip bioreactors include ability to be tailored to different areas or fields (Schipper et al., 2010) and low external energy requirements (Christianson and Tyndall, 2011).

Different organic media have been used in bioreactors for treatment of NO_3-enriched discharges from a variety of applications (Wang and Chu, 2016) with variable results. Saliling et al., (2007) used woodchips and wheat straw for treatment of aquaculture wastewater containing ≈45 mg NO_3^--N L^{-1} with denitrification rates ≈1.4 g N L^{-1} d^{-1}. Cameron and Schipper (2010) denitrified drinking water containing ≈140-160 mg NO_3^--N L^{-1} with several types of woodchips and wheat straw obtaining rates between ≈ 0.005-0.011 g N L^{-1} d^{-1}. Healy et al. (2012) treated groundwater with ≈19-32 mg NO_3^--N L^{-1} reaching denitrification rates between ≈ 0.002–0.003 g N L^{-1} d^{-1}. Feyereisen et al. (2016) removed up to ≈ 35 g N L^{-1} d^{-1} from agricultural tile drainage containing ≈50 mg L^{-1} NO_3^--N with residues from corn cobs, corn stover and barley straw. An advantage of wood media vs. non-wood media is that the wood media can have a more diverse microbial community, providing a robust microbial ecosystem with better conditions for denitrification (Grießmeier and Gescher, 2018). With respect to woodchips, softwood (e.g., pinus) and hardwood (e.g., eucalyptus) species have been used. Although it is expected that woodchips from hardwood species have a longer duration because of slower degradation (Addy et al., 2016), several studies did not find significant differences in nitrate removal rates between both types of wood (Cameron and Schipper, 2010; Manca et al., 2020).

Denitrifying woodchip bioreactors have been used in several countries, mainly in USA (Christianson et al., 2020) and New Zealand (Schipper et al., 2005). They have been included in the official nutrient reduction strategies in several states in the Midwestern United States (IDALS, 2014) and in the National Service of Natural Resources of the Department of Agriculture of the United States (USDA, 2015). However, its application in Europe is scarce and more research is required to explore the use and optimization of bioreactors for new applications (Povilaitis et al., 2018).

The aim of this work was to evaluate the performance of citrus woodchip bioreactors for denitrification of brine from groundwater desalination plants and to address a comprehensive analysis of factors involved in their operation such as redox conditions, hydraulic residence time, seasonal changes in temperature, dissolved organic carbon release and woodchip aging. To the authors' knowledge, this is the first study to look at the efficacy of citrus woodchip bioreactors for the treatment of brine from groundwater desalination plants. Citrus woodchips have only been used as carbon media for bioreactors in preliminary short laboratory trials, in which this waste showed good performance for brine denitrification (Díaz-García et al., 2020). However longer assays are necessary prior the general adoption of this technology. Hence, our results can be considered a novel contribution to the state of the art in woodchips bioreactors research.

2. Materials and methods

2.1 Site description

The Campo de Cartagena, Region of Murcia is located in the southeast of Spain and is one of the main agricultural fertigation areas of Europe. This region is characterized by a Mediterranean semiarid climate, average annual temperatures of 18 ℃, and precipitation of ~300 mm year^{-1}, leading to a severe hydric deficit (average potential evapotranspiration of 1275 mm year^{-1}) (Jiménez-Martínez et al., 2011). Despite being a dryland area, the warm temperatures throughout the year allow the zone, comprised of ≈45 000 hectares of irrigated land, to be one of the fundamental suppliers of horticulture products and citrus to Spain and central Europe. One of the most heavily used water resources among farmers in the region to maintain intensive fertigation has been groundwater withdrawal (Jiménez-Martínez et al., 2016; Díaz-García et al., 2020). However, since the aquifers are salinized (≈3.9 to ≈6.5 mS cm^{-1}, Scientific Advisory Group for El Mar Menor, 2017) and polluted by high nitrate loads (22-34 mg L^{-1} NO_3^--N according to Jiménez-Martínez et al., 2011, 2016; reaching 30-45 mg L^{-1} closer to the coast (Tragsatec, 2020), it is necessary to desalinize groundwater before using.

Desalination in the region is typically performed in small reverse-osmosis desalinization plants installed on local farms (annual withdrawals of groundwater ≈100-110 m^3; brine production ≈20-25 hm^3). Although the cost of the process is economically profitable (Aparicio, 2017), farmers are required to install a system for denitrification of the nitrate-enriched brine produced during the desalination process (Murcia Regional Government, 2018) to prevent harmful effects of NO_3^- released to water bodies.

The latter is particularly important for preservation of the Mar Menor lagoon (the largest coastal saline lagoon of the Mediterranean basin), which suffers a severe eutrophication process (Jiménez-Martínez et al., 2016; Scientific Advisory Group for El Mar Menor, 2017; Ruiz-Fernández et al., 2019). The Mar Menor and Campo de Cartagena were declared a Vulnerable Area to Nitrate Contamination under Directive 91/676/EEC, and the Mar Menor Sensitive Area subject to eutrophication in June 2001 under the European Directive 91/271/EEC (Castejón-Porcel et al., 2018). Water in the Quaternary aquifer (the shallower regional aquifer) was deemed poor chemical quality by the Spanish Government (Ministry for Ecological Transition of Spain, 2020). According to the latter, ≈360 tons of NO_3^--N were discharged to the Mar Menor by the Quaternary aquifer between 2018 and 2019. The installation of woodchips bioreactors has been one of the proposed options by the regional government for denitrification (Murcia Regional Government, 2020).

2.2 Description of the experimental set up

A 121-week pilot scale experiment was conducted at the Agri-food Experimental Station Tomás Ferro (ESEA) (N 37° 41' 17.6" and W 0° 57' 04.4") of the School of Agricultural Engineering, Technical University of Cartagena (ETSIA-UPCT), Cartagena, Region of Murcia, Spain, between November 2017 and March 2020. The pilot plant is equipped with a well (depth = 50 m) for groundwater withdrawal (Table 1), a desalination plant with reverse osmosis (capacity of 80 m^3 d^{-1}) which produces 68.7 % fresh water and 31.3 % brine by volume (Table 1) and a number of bioreactors (Figure S1, supplementary material). Brine was stored on-site in an opaque tank for 48 h at ambient temperature to ensure continuous availability for the batch experiments. Woodchip bioreactors used for the experiment described in the present paper consisted of three rectangular, above-ground fiberglass containers (142 x 108.5 x 85 cm). Each bioreactor contained a vertical PVC pipe (63 mm of diameter) placed within the woodchip media with holes at 25 cm from the bottom of the bioreactor to allow water to enter the well (Figure 1). Each bioreactor was filled with 122 kg of citrus woodchips (Table 2) which are readily available in Mediterranean coastal areas of south Europe. Two net bags (30 x 60 cm with 2 x 2 mm sized mesh), each with 1 kg of dried (at 65 °C) woodchips, were placed at 25 cm from the bottom in each bioreactor to assess weight loss of woodchips over the duration of the experiment.

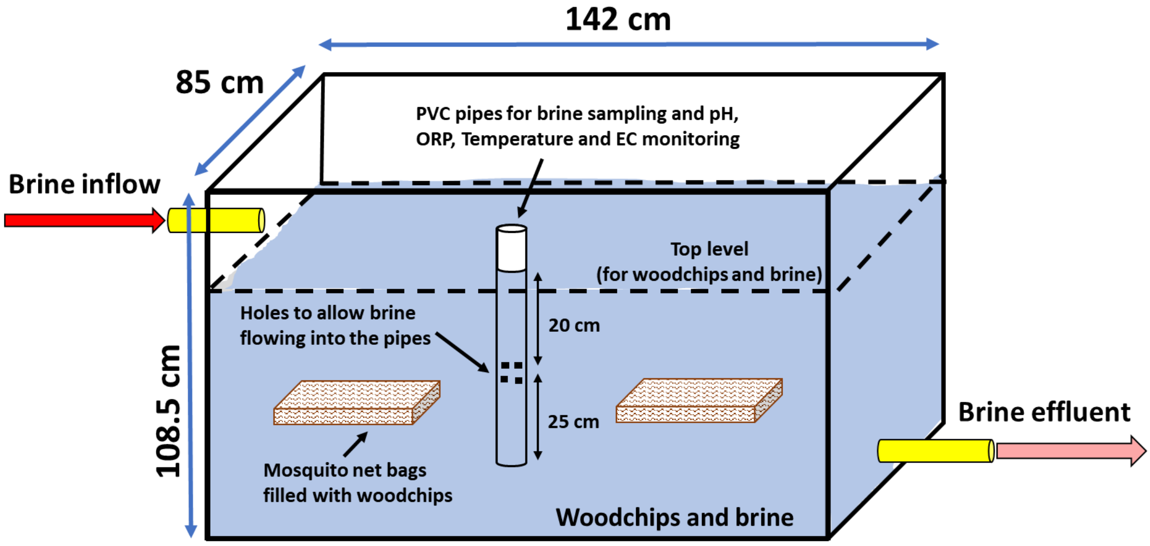


Figure 1. Scheme of the woodchip bioreactors.

Table 1. Characteristics of groundwater and the brine used in the 121-week experiment. Values are average ± standard error. Min.: minimum; Max.: maximum; EC: electrical conductivity; ORP: oxidation-reduction potential.

Parameters	Groundwater (n=48)		Brine (n=253)	
	Average	Min. - Max.	Average	Min. - Max.
pH	7.33 ± 0.03	6.88 – 7.71	7.77 ± 0.02	5.65 – 8.23
ORP (mV)	244 ± 8.7	140.1 – 381.2	231.1 ± 3.8	119.6 – 403.1
EC (mS cm^{-1})	6.2 ± 0.1	5.2 – 7.4	17.7 ± 0.1	16 - 20
NO_3^--N (mg L^{-1})	18.1 ± 0.2	14.9 – 24.8	48.5 ± 0.3	38.6 – 59.3
Cl^- (mg L^{-1})	1636 ± 15	1400 - 1847	5007 ± 33	3907 – 6967
SO_4^{2-} (mg L^{-1})	1440 ± 12	1265 - 1641	4543 ± 34	2587 - 6658
Ca^{2+} (mg L^{-1})	341 ± 2.5	303.4 – 383.5	1066 ± 5.1	793 - 1262

Mg^{2+} (mg L^{-1})	268.6 ± 2.1	236.7 - 307	858 ± 5.3	682 - 1229
Na^{+} (mg L^{-1})	969.4 ± 7.8	844.8 - 1083	3019 ± 22	2340 - 4147

Table 2. Characteristics of the citrus woodchips. EC: electrical conductivity; DOC: dissolved organic carbon. The values are the average ± standard error (n = 3). Parameters measured after flooding citrus woodchips with distilled water for two hours.

Parameter	**Data**	**Parameter**	**Data**
Average length (mm)	35.7 ± 1.7	EC (mS cm^{-1})	2.60 ± 0.25
Average diameter (mm)	5.19 ± 0.4	pH	5.69 ± 0.01
Bulk density (kg m^{-3})	230.9 ± 7.7	DOC (mg L^{-1})	2112 ± 421
Porosity (% volume)	56.6 ± 1.6	NO_3^- -N (mg L^{-1})	2.04 ± 0.07

2.3 Experimental design, monitoring, and sampling

Woodchip bioreactors were operated in batch mode with three batch runs performed each week at a 24 h hydraulic residence time (HRT). Each Monday at ≈ 8 a.m. each bioreactor was filled with brine until water level in the bioreactor was even with the surface of the woodchip media (average 242.2 ± 1.3 L of brine per bioreactor). Woodchips remained fully saturated for a 24 h period (until Tuesday 8 a.m.), after which tanks were completely drained. After removing the brine, bioreactors were immediately refilled (< 1 h after draining) and woodchips resaturated with new brine for the next 24 h batch experiment. At Wednesday 8 a.m. they were emptied and refilled again for a third 24 h batch. On Thursday 8 a.m. of each week they were emptied for the third time, after which the woodchip media remained unsaturated without brine for 96 hours until starting the next 24 h batch on Monday of the following week. This mode of operation was designed based on expected operational guidelines for farmers in the Campo de Cartagena. In total, 765 batches were performed. A total of 186 m^3 of brine were denitrified during the experiment. Denitrified brine was stored in a detention basin for further management and off-site disposal.

From weeks 1 to 96, bioreactors were monitored and sampled during each of the three weekly batches. From week 97 onwards, bioreactors were monitored and sampled only during the second weekly batch (on Tuesday), although three batches were still performed each week. Samples of groundwater were collected from the well every two weeks (total samples 48). Samples of brine were collected each day prior to filling the bioreactors (total samples 253) (Table 1). After saturating the woodchips, brine pH, temperature, electrical conductivity (EC) and oxidation-reduction potential (ORP) were measured by inserting a calibrated multiparameter instrument (Hanna HI 98194 pH/EC/DO Multiparameter) within the vertical PVC sampling well. These measurements were made at 30 min, 10 h and 24 h of HRT. Values for ORP were adjusted according to Vepraskas and Faulker (2001), by adding +200 mV to the measured values (the voltage of the Ag/AgCl reference electrode at 20 °C). During sampling of the water in the bioreactors, brine within the vertical PVC sampling pipes was first vacated using a polyethylene (PE) sampler, allowing the pipe to refill with brine in contact with the woodchips, prior to taking *in situ* measurements and collecting samples. Brine samples were collected at 10 h HRT by extracting water from the vertical PVC pipes using the PE sampler, and at 24 h HRT from the outlet of the bioreactors. Samples were filtered through Microsart CN-Filter filters (0.45 µm pore size). Additionally, one of the woodchip-filled net bags was removed from each bioreactor at 12 and 24 months after the experiment began. Bags were oven dried at 65 °C until constant weight and weighed to assess woodchip loss.

2.4 Analyses

Woodchip characterization. Bulk density and porosity of the woodchips were measured according to Christianson (2010). For bulk density, 2 L containers were filled with woodchips and weighed to calculate the mass:volume ratio. Then, distilled water was added over 2 h filling the drainable porosity and internal pore space of the woodchip, where total volume of distilled water added was considered the effective porosity. Aliquots of the distilled water were collected after the 2 h and analyzed for EC, pH, dissolved organic carbon (DOC) (carbon analyzer TOC-V CSH Shimadzu), and NO_3^--N (double channel chromatographic system 850 Professional Ion Chromatography y Metrohm).

Well and brine sample analyses. Samples of well water and brine were analyzed for NO_3^-, NO_2^-, Cl^-, SO_4^{2-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, with a double channel chromatographic system 850 Professional Ion Chromatography Metrohm. Samples from each of the three weekly batches were analyzed during first 53 weeks (first 12 months), while samples from the second batch (Tuesday) each week were analyzed during the rest of the experiment, with 1312 samples measured in total. DOC concentration was only measured for samples collected from the second batch of each week (Tuesday) throughout the experiment (583 samples were analyzed; carbon analyzer TOC-V CSH Shimadzu). All water chemistry analysis was performed at the Technological Research Support Service (SAIT) of the Technical University of Cartagena.

In order to evaluate the performance of the bioreactors, Nitrate Removal Efficiency (NRE) and NO_3^--N Removal Rates (RNO_3) were calculated for each batch run according to Christianson et al. (2015). (Eq. 1):

$$R_{NO_3}(\mathrm{g\ N\ m^{-3}\ d^{-1}}) = \frac{(\mathrm{NO_3^- {-} N\ influent\ concentration} - \mathrm{NO_3^- {-} N\ effluent\ concentration})\ x\ V_{water}}{V_{saturated\ woodchips}\ \ x\ t} \quad (1)$$

Where influent concentration was the NO_3^--N in the initial brine of each batch (g N m^{-3}), effluent concentration was the NO_3^--N in the effluent after 24 hours (g N m^{-3}), V_{water} was the volume of water in each bioreactor, $V_{saturated\ woodchips}$ was the volume of saturated woodchips (m^3) and t was the time of the nitrate measurement (d).

Measured values of RNO_3 and water temperature inside the PVC well were used to calculate Q_{10}, whose values were used as a metric for temperature sensitivity of nitrate removal Q_{10} is defined as the factor by which a rate of reaction increases for each 10 °C increase in temperature. Data were fitted to equation (2) and equation (3) to calculate Q_{10} values. Collected data was fitted to the relationship in equation (2) using the nls() function in R Studio (RStudio, 2020) a function finding the least-squares parameter estimates of a nonlinear function, solving for R and k (Maxwell et al., 2020a).

$$R_T = R_0\ xe^{kT} \quad (2)$$

$$Q_{10} = e^{10\ x\ k} \quad (3)$$

2.5. Statistics

Statistical analyses were performed with IBM SPSS Statistics 19.0.0 (SPSS, 2010). Pearson correlations was used to check relations among variables. Non-parametric Friedman rank test followed by Dunn's multiple comparison post hoc test was used to identify differences in NRE among different seasons (periods with different average temperature). One-way ANOVA was used to compare woodchip weight loss at months 0, 6 and 12. Differences were considered significant at $p < 0.05$. Data are shown as average ± standard error throughout the paper.

3. Results

3.1 Physicochemical conditions (ORP and pH)

Between weeks 1 to 53 (≈ 1st year, middle Fall 2017-middle Fall 2018) the variability in ORP between the three bioreactors was higher than from 54th week onwards (middle Fall 2018-Winter 2020), mainly at 30 min and 10 h after saturation, as shown by the higher standard error values during the first period (Figure 2). Additionally, ORP values and their seasonal variations differed between the two periods mentioned.

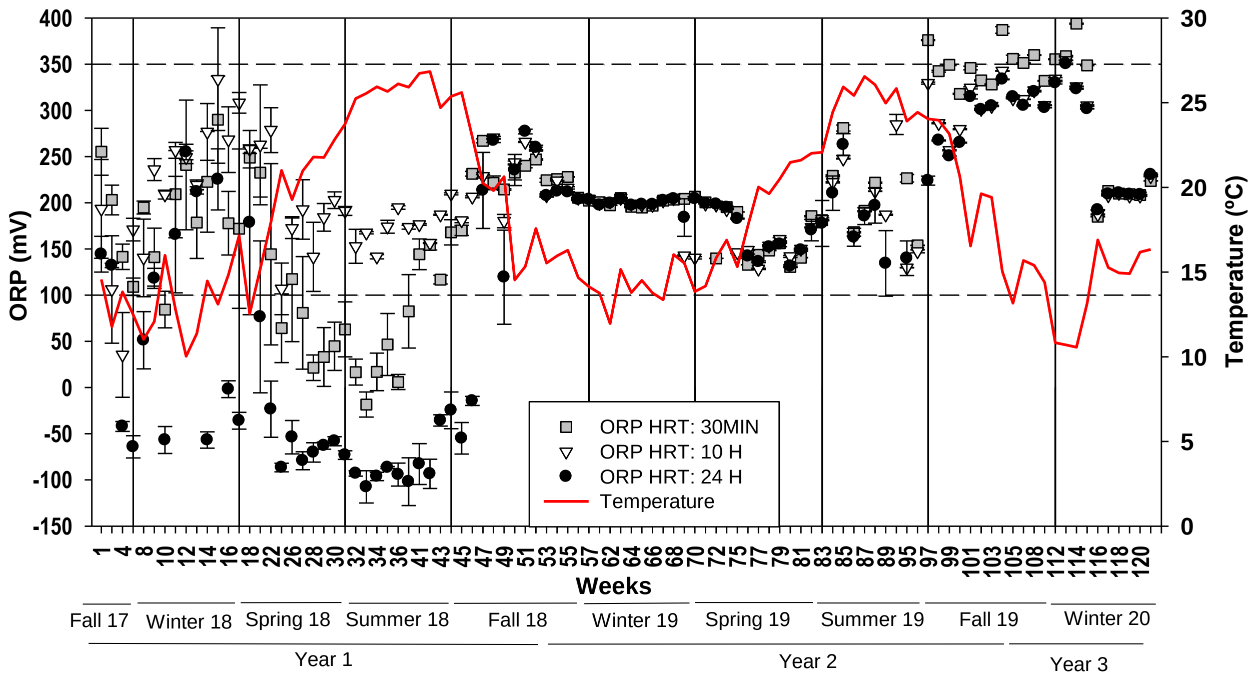


Figure 2. Weekly average of Oxidation Reduction Potential (ORP) at 30 min, 10 h and 24 h HRT, and temperature, inside the bioreactors, for the 121 weeks of the experiment. Values are the average ± standard error. Weeks from 1 to 96, n = 9 (three sampling days with three repetitions per day); weeks from 97 to 121, n = 3 (one sampling day with three repetitions). HRT: Hydraulic Residence Time. Dashed lines show the range of suboxic conditions considered optimal for denitrification (ORP between +100 and +350 mV) (Otero and Macías, 2003).

During the first year, the ORP at 30 min HRT fluctuated from ≈ +150 to ≈ +350 mV when average temperatures was ≈ 10 °C (weeks ≈ 1 to ≈ 18 and ≈ 43 to ≈ 53) (Figure 2). When temperature increased (weeks ≈ 18 to ≈ 41) ORP values decreased reaching ≈ 0 mV. ORP at 10 h HRT also tended to decrease at warmer temperatures, although the drop was less pronounced than at 30 min HRT (ORP was always between ≈ +150 to ≈ +350 mV). Finally, during the first year ORP at 24 h HRT was almost always between ≈ -100 and 0 mV, regardless of temperature.

ORP behavior was different during the second year of operation, relative to the first year (Figure 2). Between ≈ 53 and ≈ 83 weeks (temperature ≈ 14 - ≈ 17 °C), ORP was fairly stable and similar among the three HRT (≈ +150 to ≈ +200 mV), but when temperature increased (> ≈ 25°C) some distinction was observed (weeks ≈ 83 to ≈ 97) although values were always between ≈ +150 and ≈ +250 mV. During ≈ 97 to ≈ 114 weeks the ORP increased up to ≈ +350 - ≈ +400 mV at 30 min HRT and up to ≈ +300 mV at 10 h and 24 h HRT. Finally, from week ≈ 115 onwards the ORP values at the three HRT were similar (≈ +20 mV).

In contrast to ORP, pH inside the bioreactors appeared relatively constant during the 121 weeks of the experiment, with an average pH of 7.60 ± 0.007 at all HRT (n = 1689; data not shown). EC values were also stable over the experiment and during batches, with an average value of 17.74 ± 0.05 mS cm^{-1} (n = 1452; data not shown).

3.2 DOC and NO_3^--N concentrations

On the first batch run of the first week of the study period (1-1 in Figure 3), DOC concentrations (Figure 3) reached ≈ 1567 mg L^{-1} at 10 h HRT. By the third batch run of the first week (1-3 in Figure 3), DOC concentrations had dropped to ≈ 300 mg C L^{-1} and decreased further to ≈ 10 mg C L^{-1} between weeks ≈ 8 to ≈ 17 (winter 2018). From weeks 18 to 43 (spring and summer 2018), when temperatures rose, DOC increased again to ≈ 15 - 20 mg C L^{-1} at 10 h HRT and ≈ 30 - 40 mg C L^{-1} at 24 h HRT. Between weeks ≈ 43 and ≈ 108 (second year, fall 2018 – fall 2019) DOC concentrations were ≈ 10 - 16 mg C L^{-1}, regardless of HRT and temperature, and from week ≈ 109 (winter third year) until the end of the experiment DOC was relatively stable (≈ 7 mg C L^{-1}).

Woodchip mass remaining (average ± SE) inside the net bags relative to the initial weight was significantly lower at six (≈69% of initial weight) and twelve (≈58% of initial weight) months (Table S1). This meant a weight loss ≈31 % between months 1 and 6, and ≈11 % between months 6 and 12.

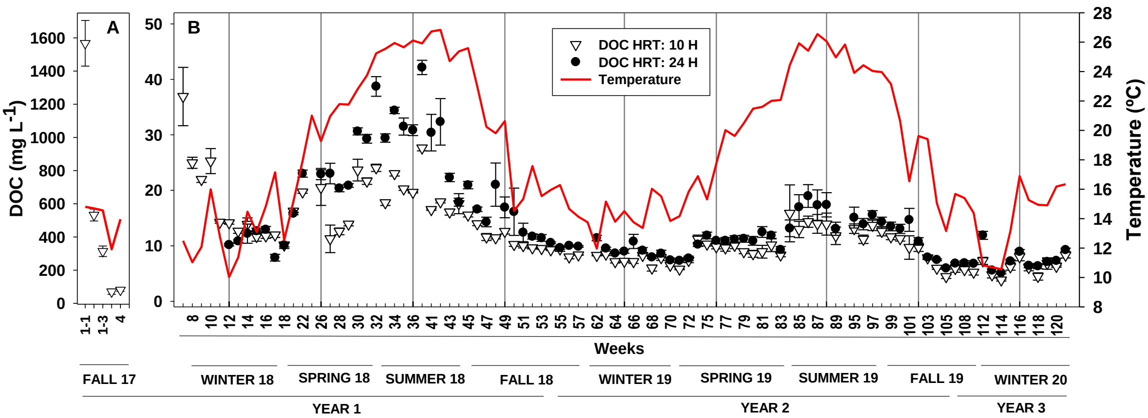


Figure 3. Weekly average of Dissolved Organic Carbon (DOC) at 10 h and 24 h HRT, and temperature, inside the bioreactors, for the 121 weeks of the experiment. A) DOC in the first five weeks (during the first week data are shown as a daily basis, from the first day, 1-1, to the third day, 1-3). B) DOC from weeks 6 to 121. Note the difference in the range of the Y axis for A and B. Values are the average ± SE. Weeks from 1 to 96, n = 9 (three sampling days with three repetitions per day); weeks from 97 to 121, n = 3 (one sampling day with three repetitions). HRT: Hydraulic Residence Time.

NO_3^--N concentrations in the inflow were between 40 and 50 mg NO_3^--N L^{-1} throughout the 121 weeks of the experiment (Figure 4A). During the first ≈ 17 weeks NO_3^--N in the effluent was ≈ 10 - 30 mg NO_3^--N L^{-1} at 10 h HRT (NRE ≈ 40 - 90 %, Figure 4B) and < ≈ 10 mg L^{-1} at 24 h HRT (NRE ≈ 80 - 95 %, Figure 4B), but between weeks ≈ 18 - 45 it was < ≈ 6 mg NO_3^--N L^{-1} at both HRT (NRE > ≈ 80%). During weeks ≈ 49 to ≈ 75, effluent NO_3^--N concentrations increased to ≈ 20 - 30 mg NO_3^--N L^{-1} at 10 h (NRE ≈ 35 - 50 %) and 24 h (NRE ≈ 50 - 70 %), coinciding with temperature decreased, and between weeks ≈ 75 - ≈ 89 decreased again at ≈ 13 mg NO_3^--N L^{-1} (10 h HRT, NRE ≈ 65 %) and ≈ 3 mg NO_3^--N L^{-1} (24 h HRT, NRE ≈ 95 %). After week 89, NO_3^--N gradually increased to 35 to 45 mg NO_3^--N L^{-1} at both HRT (NRE ≈ 25 - 40 %), when temperatures dropped to ≈ 11 - 16 °C. Nitrate Removal Rate (RNO_3) was similar at both HRT throughout the 121 weeks of experiment (Figure 4C) and ranged from ≈ 15 to 25 g N m^{-3} d^{-1} between weeks 1 to ≈ 104 (fall 2017-middle fall 2019), and from ≈ 5 to 10 g N m^{-3} d^{-1} between 105 to 121 weeks (middle fall 2019-winter 2020).

Nitrite concentrations were low throughout the experiment (average ± SE): 0.328 ± 0.03 mg NO_2^--N L^{-1} in the inflow brine (n= 269); 0.769 ± 0.03 mg NO_2^--N L^{-1} in the effluent at 24 h HRT (n=669). Hence, this analyte is not further discussed.

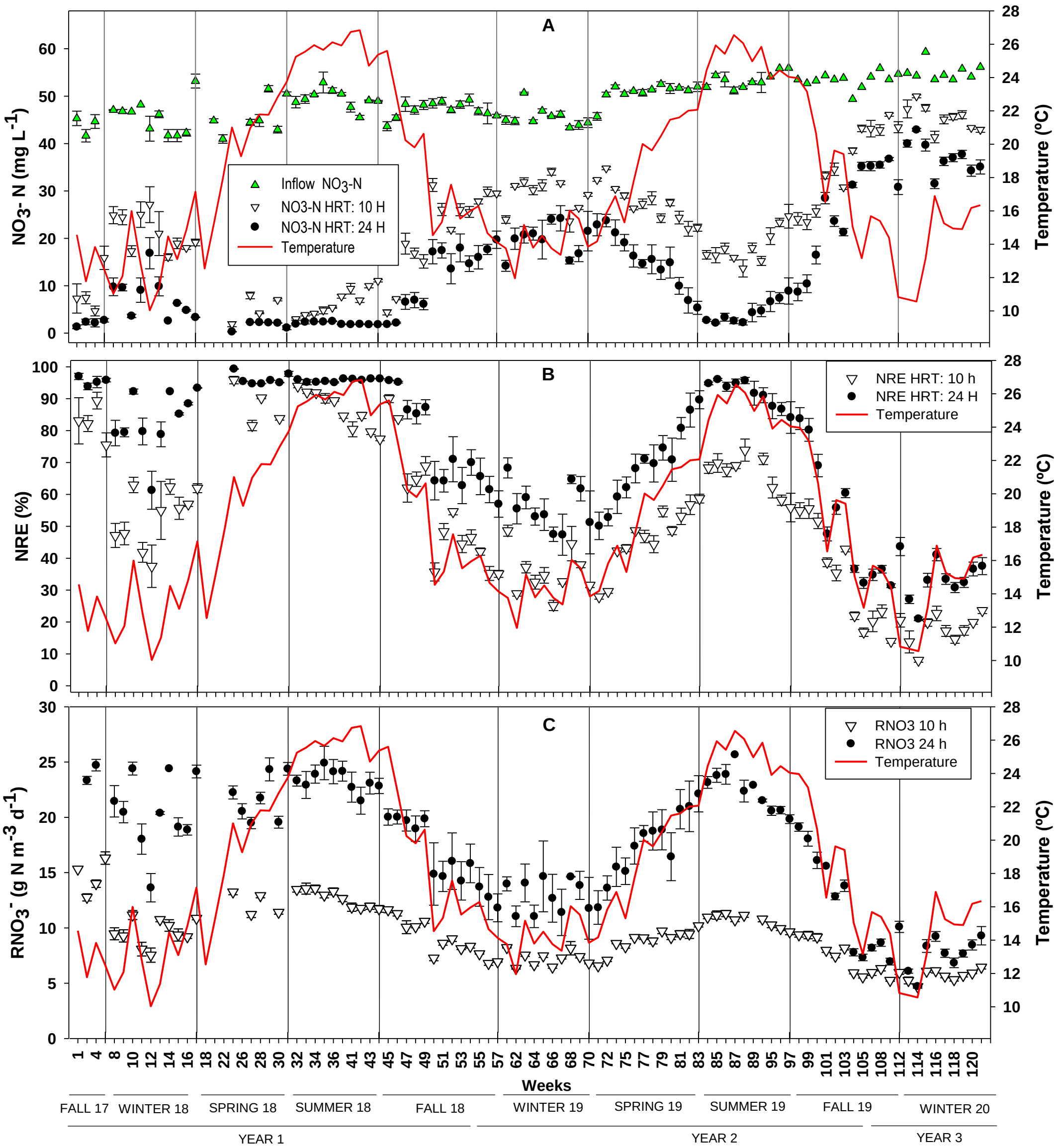


Figure 4. A) Weekly average of nitrate (NO_3^--N) concentration in the inflow and in the effluent at 10h and 24h HRT, and temperature inside the bioreactors. B) Weekly average of Nitrate Removal Efficiency (NRE). C) Weekly average of Nitrate Removal Rates (RNO_3), and temperature, in the bioreactors. Values are the average ± standard error. Weeks from 1 to 96, n = 9; weeks from 97 to 121, n = 3. HRT: Hydraulic Residence Time.

NRE and RNO_3 data were separated by season according to average temperature (Table S2) and NRE values were compared using the non-parametric Friedman test, which showed significant differences in NRE between seasons, (Table S3) with different results for 10 h and 24 h HRTs. At 10h HRT, average NRE for weeks 99 – 121 (Fall – Winter 19-20) were significantly lower than any of the other four periods. However, for 24 h HRT, there was only a significant difference in NRE between weeks 17 – 43 (Spring – Summer 18) and weeks 99 – 121 (Fall – Winter 19-20).

4. Discussion

Since denitrification is a biological process, NRE and RNO_3 are strongly influenced by physical-chemical and chemical conditions in the bioreactor (Fiedler et al., 2007; Reddy and DeLaune, 2008a; Robertson, 2010; Li et al., 2017; Ghane et al., 2018). For instance, the pH and ORP are two key parameters influencing and affected by microbial activity (Reddy and DeLaune, 2008; Tercero et al., 2015), but others such as organic carbon quantity and quality, salinity and temperature also play a role. Most of the previous factors are difficult to control directly, but others such as HRT and flooding-drying regime can be handled relatively easily during bioreactor operation.

4.1. Changes in pH, ORP and EC in the bioreactors

In the bioreactors, the pH was relatively stable with low variability throughout the 121 weeks (average of 7.6 ± 0.007)), falling within the range of pH that is known to be suitable for denitrification (pH ≈5.5 – 8) (Rivett et al., 2008; Albina et al., 2019). In flooded systems, an increase in pH is usually expected as ORP decreases due to H^+ consumption (Stumm and Sulzberger, 1992). Moreover, denitrification produces alkalinity, which often increases pH (Reddy and DeLaune, 2008). However, in this experiment a slight decrease of pH (average ≈ 0.5) was observed in the effluent at 24 h HRT relative to the initial brine, as also observed by Robertson and Merkley (2009) and Warneke et al. (2011). This decrease of pH during flooding led to positive correlation between pH and ORP ($p \leq 0.001$; Table S4) and could be due to several factors, such as the dynamics of the CO_2-H_2CO_3 system and N nitrification during drying phases (Reddy and DeLaune, 2008; Tercero et al., 2015). The CO_2 produced during mineralization of the carbon could have dissolved in the flooding water and formed H_2CO_3, a weak acid that contributed to the drop of pH observed. Furthermore, the organic acids released from the woodchips during flooding could also contribute to the decrease of pH (Albina et al., 2019), as shown by the negative correlation between DOC and pH ($p<0.001$; Table S4).

Although the microbial activity was not directly evaluated in this work, ORP is an indicator of the activity of both aerobic and anaerobic microorganisms (Fiedler et al., 2007). In well-aerated systems, where microorganisms use free oxygen for their metabolism, ORP values were > ≈ +350 mV (oxic conditions at pH ≈ 7, (Vepraskas and Faulker, 2001; Otero and Macias, 2003; Reddy and DeLaune, 2008). In flooded systems, when oxygen concentration falls below ≈ 4 % (ORP ≈ +350 mV), microorganisms use other electron acceptors (e.g., nitrate) for organic matter mineralization via anaerobic pathways and ORP decreases accordingly. The cited authors indicated that, at pH ≈ 7, a drop of ORP at values < ≈+350 mV indicates suitable conditions for denitrification. In fact, a negative correlation was found between ORP and NRE ($p \leq 0.001$; Table S4). ORP values < ≈ +100 mV indicate that sulfate (SO_4^{2-}) reduction to sulfide (S^{2-}) may occur. Since SO_4^{2-} content in the brine was high (≈ 4475 mg L^{-1}), the ORP values between +100 and -100 mV measured at 30 min and 24 h HRT during the first 41 weeks indicate potential environmental risks due to sulphate reduction. Dissolved S^{2-} is highly toxic for biota (Reddy and DeLaune, 2008), and would be an issue if bioreactor effluents are discharged into natural water bodies. It may be necessary to regularly monitor bioreactors treating brine with high SO_4^{2-} concentrations and manage the HRT to avoid ORP conditions leading to formation of these compounds. Reduced sulfur in bioreactor effluents could be managed using a complementary system with capacity to remove S^{2-}, such as a constructed wetland (Vymazal, 2014). Furthermore, a combination of both systems has been shown to have additional advantages for improving the performance and resilience of water treatment under shock loading events of other key contaminants such as TSS, BOD5 and TN (Sukias et al., 2018).

During the first ≈ 24 - 26 weeks (until mid-spring 2018) there was high variability in ORP values at 30 min and 10 h HRT. This could have been due to the start-up period of the bioreactors, where physical, biogeochemical or microbiological properties in the woodchip media had not yet stabilized. Porosity was variable as woodchips were settling, woodchips were possibly less uniform in their nutrient content, and microbial community not fully established. Low temperatures in week ≈ 30, which ranged from ≈ 10 °C to ≈ 15 °C, may have also contributed to the variability found. In a mesocosm study mimicking eutrophic wetlands, Tercero et al. (2015) found that at this temperature range microbial activity was disadvantaged and more irregular than at higher temperatures. Moreover, a negative correlation between ORP and temperature ($p \leq 0.001$) indicated that ORP drop was favored by temperature increase.

Between weeks ≈30 to ≈48 the ORP values at 30 min HRT were lower than at 10 h HRT. This may seem contradictory if we expect that in flooded systems O_2 is progressively depleted as a consequence of microorganism's activity. If so, the longer flooding time, the less oxygen content is expected. However, the results obtained may be explained as follows. If at the beginning of each batch some anoxic brine from the previous batch remained in the pores of the woodchips, this previously denitrified brine could cause the sharp drop in ORP observed at 30 min HRT. When the conditions of the new brine (which introduced O_2 and NO_3^-, two oxidants) were prevalent, the ORP would have increased and stabilized to a certain level, as reflected by the values obtained at 10 h (≈ +150 to +200 mV). Later, O_2 consumption by microorganisms led to ORP drop again until reaching values indicative of anoxic conditions (< +100 mV) at 24 h HRT. From week ≈ 49 onwards ORP variability decreased, which suggests that the system was physically (e.g., pore spaces) and microbiologically (e.g., microorganisms' population) more homogeneous.

Research about the role of salinity in denitrification has provided variable and even contradictory results. Lay et al. (2010) found that salinity decreased denitrification by affecting microorganisms in maintaining their osmotic pressure balance. Other studies have presented that high salinity could cause the inhibition of denitrification (Marton et al., 2012; Trögl et al., 2012). von Ahnen (2019) found that high salinity altered the woodchip microbiome leading to a drop in denitrification, and Dinçer and Kargi (2000) that high salt contents adversely affect nitrification and denitrification of saline wastewater. However other researchers did not find apparent drawbacks for denitrifying microorganisms in saline environments (Reddy and DeLaune, 2008; Trögl et al., 2011; Álvarez-Rogel et al., 2016).

The present experiment was not designed to evaluate the effect of salinity on RNO_3 and NRE. Despite this, high nitrate removal was consistently observed throughout the 121 weeks experiment, even if rates were not constant, while salinity of the initial brine was high and fairly stable throughout (≈ 17 dS m^{-1}). Salinity was not significantly correlated with NRE or other parameters (Tables S4). Furthermore, Maxwell et al. (2020b) found an increase in RNO_3 when salinity of brine increased in bioreactors with similar woodchips, although the experiment was performed over a shorter duration (9 weeks). The positive effect of salinity on denitrification could be due to the indirect effect of higher salinity increasing the breakdown of organic matter (Weston et al., 2011; Marton et al., 2012), thereby increasing easily available substrate for microbial activity. For instance, Steele and Aitkenhead-Peterson (2013) showed that organic carbon leaching from senesced vegetation remains increased with sodicity due to the interaction of sodium ions with organic functional groups that increase their solubility. In addition, the high salinity of brine could lead to strong osmotic potential gradient between internal pores of woodchips and the macropore water, could have much contribute to DOC accumulation, with initial diffusion being a major driver. In fact, other experiments at the UPCT facility showed DOC in the effluent of woodchips was greater as brine became more concentrated (Maxwell et al., 2020a).

4.2. Seasonal changes in temperature and bioreactors performance

Temperature accelerates microbial metabolism (Bell et al., 2015; Manca et al., 2020) and hence stimulation of denitrification at higher temperature is expected. This effect was not apparent during the first ≈47 weeks of experiment, when ORP, NRE and RNO_3 were hardly affected by changes in temperature, mainly at 24h HRT. However, temperature had a large effect on the bioreactor's performance as the experiment progressed and become a decisive factor from week ≈47 onwards, when these three parameters began to oscillate following temperature oscillations (Figures 2, 3 and 4A). Moreover, temperature and NRE were the factors that showed the strongest correlation for the whole experiment ($r=0.511$; $p<0.001$; Table S4).

The temperature dependence of nitrate removal was also observed by other authors such as Halaburka et al. (2017), that found that temperature explained 50 % of the variability in woodchip denitrification rates. Addy et al. (2016) summarized several published studies about denitrifying bioreactors in which RNO_3 increased at higher temperature ranges. They reported RNO_3 values between ≈ 2.1 and ≈ 5.7 g N m^{-3} d^{-1} at a temperature range between ≈ 6 and ≈ 17 °C and RNO_3 values ≈ 8.6 g N m^{-3} d^{-1} at temperature > ≈ 17 °C. Von Ahnen et al. (2016a) obtained RNO_3 values between 6.24 and 8.40 g N m^{-3} d^{-1} with a temperature between 7.0 to 9.6 °C, and Greenan et al. (2009) RNO_3 values between 2.9 and 4.5 g N m^{-3} d^{-1} with a temperature of 10 °C. In our experiment we found an average

RNO_3 of 18.9 ± 0.72 g N m^{-3} d^{-1} (maximum 37.4 g N m^{-3} d^{-1}) with a daily average temperature of 18.3 ± 0.54 °C, similar to values obtain by Hoover et al. (2015), who at 20 to 21.5 °C reached a RNO_3 between 10 - 21 g N m^{-3} d^{-1}. By contrast, Warneke et al. (2011) reached an average of 7.63 ± 0.88 g N m^{-3} d^{-1} with temperatures between 15.5 and 23.7°C, with the highest RNO_3 of 11.2 g N m^{-3} d^{-1} at 23.7 °C.

The cited studies show the importance of temperature for woodchips denitrifying bioreactors performance and point that these systems would be well-suited to warm climates such as southeastern Spain. A higher dependence of RNO_3 on temperature as woodchips age has been explained by the lower quality of organic carbon produced from woodchips (Robertson, 2010; Xu et al., 2012). In this experiment, values of the Q_{10} coefficient were 1.06 ± 0.021 between weeks 1 and 53 (RNO_3 ≈ 21 g N $m^{-3}d^{-1}$ y NRE ≈ 88 %), and 1.77 ± 0.067 between weeks 54 and 121 (RNO_3 ≈ 16 g N $m^{-3}d^{-1}$ y NRE ≈ 66 %), showing a greater dependence on temperature during the second period than in the first one when woodchips were fresh, in agreement with Maxwell et al. (2020a).

4.3. DOC, woodchips longevity, flow regime and HRT

Another important factor for bioreactor performance is the longevity of woodchips (Moorman et al., 2010). Weight loss of the woodchips was higher in the first year than in the second (≈ 31 % and ≈ 11 %, respectively). During the first year of bioreactor operation, with fresh woodchips, microorganisms had greater access to labile carbon (high cellulose content). As the woodchips progressively aged, the quantity and quality of DOC would have decreased, with the woodchips becoming more recalcitrant and therefore more difficult for its rapid consumption by microorganisms (Masbough et al., 2005; Maxwell et al., 2020b). The weight losses found in this work were higher than those reported by Schipper and Vojvodić-Vuković (2001) and Moorman et al. (2010) after 5 and 9 years respectively.

Longevity of woodchips affects bioreactors cost. Christianson et al. (2021) collected several studies in which this aspect was analyzed. The most used rate is in terms of dollars per kg of nitrate removed. Schipper et al. (2010) found a range in initial cost of $2.40 to $15.20/kg N denitrified, and these values were corroborated in recent estimations (Christianson et al., 2021). In this study 2.7 kg N were removed during the experiment (121 weeks). Considering a cost of $5.10 in woodchips to fill each bioreactor, the final cost was $1.89 /kg N denitrified. However, since woodchips were not depleted the bioreactors could be working for longer time and so the final cost per kg N denitrified would be lower than $1.89 /kg N denitrified.

The flow regime is a key factor influencing woodchips degradation. In the experiments of Schipper and Vojvodić-Vuković (2001) and Moorman et al. (2010), the bioreactors were operated under continuous flow, while those in the current study were done in batch mode. Woodchips in these batch experiments also remained unsaturated for a period of four days empty (from Thursday to Monday). These phases of drying and rewetting have been shown to promote greater degradation of woodchips via aerobic breakdown since aerobic decomposition is normally more efficient than anaerobic (Moorman et al., 2010; Maxwell et al., 2018). For that reason, denitrifiers would have had greater access to more labile carbon immediately following unsaturated periods that made lower molecular weight carbon more available via aerobic processes (Maxwell et al., 2020a). These drying-rewetting cycles also increase carbon leaching, with DOC content being higher at the beginning of the flooding phases but decreasing quickly (i.e., within a matter of days) upon resaturation as aerobically-produced carbon is leached or consumed by microbes as a result of the more rapid aerobic degradation (Hansson et al., 2010; Maxwell et al., 2018). This gradual leaching/loss of labile carbon was reflected in our experiment by the progressive decrease of NRE from Monday (just after four days of bioreactors drying) to Wednesday (the third consecutive weekly flooding batch) (Figure 5). The loss of more labile carbon over time would also explain the downward trend of NRE and RNO_3 over the 121 weeks experiment. DOC production from woodchips could occur at irregular pulses inside bioreactors and not in a homogeneous way, until those woodchips of different shapes and sizes were settled, and pore space conditions were homogenizing. Moreover, quantity and quality of DOC (an issue discussed below) could be more variable during the first months when woodchips were more heterogeneous, and some pieces could be more prone to provide easily metabolizable carbon than others.

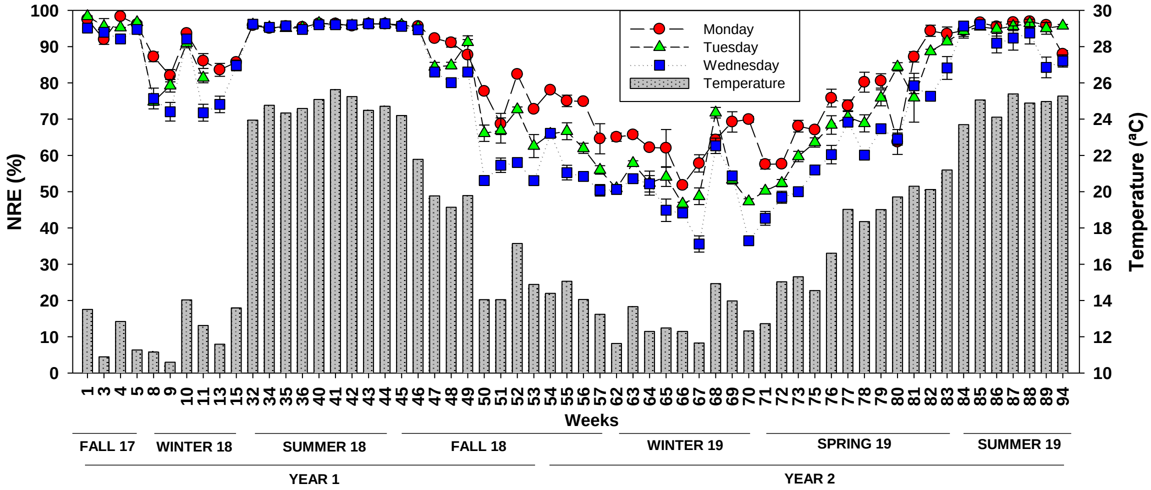


Figure 5. Daily average of Nitrate Removal Efficiency (NRE) in the effluents at 24 h HRT of Monday, Tuesday and Wednesday and temperature inside the bioreactors. Values are the average ± standard error. Weeks from 1 to 94 (n = 3). HRT: Hydraulic Residence Time.

The decrease in quality/quantity of DOC over time may explain differences in how nitrate removal responded to temperature changes. Contrasting with the warm period (temperature > ≈ 20 °C) of 2018 (weeks ≈ 18 to 44), when temperature increased in 2019 (weeks ≈ 79 to 100) the ORP did not decrease lower than +100 mV, possibly due to the lower quality (more recalcitrant) of the DOC available that hindered microbial activity in some way. Later, the drop in temperature between ≈ 100 and ≈ 112 weeks (fall 19-early winter 2020) combined with the low DOC concentrations (< 10 mg L^{-1}) may have been the cause of lower N removal rates and the observed rise in ORP. Robertson (2010) and Maxwell et al. (2020a) indicated that the influence of temperature on microbial activity become more important in aged woodchips and attributed this behavior to the worse media quality together with the more difficult for microorganisms to work under cold conditions. The positive effect of temperature increase on microbial activity was shown by the drop in ORP at all three HRT, from week ≈ 116 onwards, when temperature rose up to 15 °C. This effect was observed for other studies, due to temperature accelerates microbial metabolism (Bell et al., 2015; Manca et al., 2020).

Lastly, the HRT is another key factor for nitrate removal performance, since it must be long enough for the microorganisms to carry out the denitrification process, obtaining the necessary energy through solubilization and consumption of the organic substrate (Addy et al., 2016). During periods of greater microbial activity (e.g., warm temperatures), a lower HRT would be necessary to achieve full denitrification of the water. Robertson and Blowes (2000) (in a pilot-scale drainage with an inflow of 4.8 mg NO_3^--N L^{-1}, 1.9 m^3 bioreactor and a temperature between 2 to 20 °C) and Christianson and Helmers (2011) (in a field scale drainage with an inflow between 7.03 to 13.11 mg NO_3^--N L^{-1}, 102 m^3 bioreactor and a temperature between 3 to 15 °C) concluded that an HRT < 8 h was enough to achieve NRE of ≈ 60 %. By contrast, Greenan et al. (2009) (in a laboratory scale drainage with an inflow between 50 mg NO_3^--N L^{-1}, 0.01 m^3 bioreactor and an average temperature of 10 °C) needed almost 4 days to reach the same efficiency when also treating agricultural drainage. The data obtained in our experiment show that in the first year (≈ 48 weeks), 10 h HRT was enough to remove most of the NO_3^--N (NRE ≈ 75 %) in the brine (in many occasions comparable to NRE seen at 24 h HRT), but from week ≈ 49 onwards (beginning of the second year) the NRE at 10 h HRT decreased and was lower than NRE at 24 h HRT until week 121 (end of the experiment), regardless of temperature. This effect was exacerbated in Fall 19 – Winter 20 (weeks 99 – 121), when NRE were significantly lower than in previous seasons. The high NRE values (> 80 %) during the first ≈ 48 weeks at 24 h HRT even in colder periods could have been caused by the initial DOC flush from the fresh woodchips and would explain why denitrification was not as affected by temperature due to the high availability of organic carbon for microorganisms. This is consistent with Brettar et al. (2002), who saw high nitrate reduction coupled with high availability of organic matter and low ORP, with nitrate removal mostly independent of temperature.

5. Conclusions and guidelines for management

Our results showed that citrus woodchip bioreactors are a suitable option for denitrification of nitrate-enriched brine despite its high salinity. In the Campo de Cartagena, the warm climate would favor high N removal efficiency in these systems operating at 24 h HRT, at least during the first 94 weeks (≈ first 2.5 years of bioreactors operation). The high DOC availability (> 25 mg C L^{-1}) in the citrus woodchips during the first months (first 48 weeks) resulted in high NRE (> 75 %) even at 10 h HRT. While this higher NRE during the initial weeks does not represent the long-term N removal performance, using fresh woodchips could be used as a means for achieving high NRE even at low HRT. Use of fresh woodchips would have its own drawbacks since an excess of DOC in the effluent may present challenges during discharge, particularly if discharge limits exist for organic carbon. Prior washing of fresh woodchips could be used to reduce the risk of high DOC in early denitrified brine, separating this early DOC leaching period from the brine denitrification. This washing could be done with freshwater produced by desalination while still using the leachate-rich discharge for crop irrigation.

The extremely low ORP (< 0 mV) values reached during the first months, even at 10 h HRT, indicate that sulfide formation must also be considered during brine denitrification, due to the high sulphate content of this waste. HRT must be managed to avoid significant production of reduced sulfur, mainly during the first months in which DOC leaching from woodchips is extremely high and strong anoxic conditions are expected in the bioreactors. Monitoring of ORP can help manage this issue and reduce likelihood of sulfide formation.

Although citrus woodchips provided enough organic matter for denitrification even after 2.5 years (as shown by RNO_3 reaching 9.3 g N m^{-3} d^{-1} in week 121), the effect of temperature on NRE became more apparent from week ≈49 onwards (second year). This was likely caused by gradual woodchips degradation upon successive washing and indicates that, even under warm climate conditions, maintaining high NRE of the bioreactor requires active management. One option to improve performance is based on the fact that nitrate removal efficiency was highest on Mondays (first weekly batch), immediately after woodchips had been unsaturated for four days. During these four days of unsaturated conditions, it is assumed that aerobic microbial metabolism produced a flush of DOC that stimulated denitrification on the first day following re-saturation for the media (Monday in our experiment).

Developing strategies for implementing a drying-rewetting regime could improve the nitrate removal performance, particularly in colder seasons with aged woodchips. Use of in situ nitrate sensors could allow water quality managers to determine when sufficient NRE has been achieved and water should be discharged to the system. Less costly ORP sensors could be used instead to detect nitrate depletion and potential sulfide formation. Use of this monitoring would need to evaluate for each specific bioreactor since they are expensive and relatively difficult to manage.

Contributor roles taxonomy (CRediT) statement

Carolina Díaz-García: Conceptualization, writing – original draft, methodology, review and editing, investigation, data curation, visualization, formal analysis. **Juan José Martínez-Sanchez**: Conceptualization, methodology, investigation, resources, supervision, project administration, formal analysis, and funding acquisition. **Bryan M. Maxwell:** Conceptualization, review and editing, and formal analysis. **José Antonio Franco:** Conceptualization, review, and supervision. **José Álvarez-Rogel**: Conceptualization, investigation, methodology, supervision, formal analysis, review, and editing.

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.

Acknowledgements

This study was supported by the Chair of Sustainable Agriculture for the Campo de Cartagena (Cátedra de Agricultura Sostenible para el Campo de Cartagena, https://www.catedraagriculturasostenible.es/). The authors thank Ana Belén Rodríguez, Ana Vanessa Caparrós, Pedro Antonio Madrid and Cristian Molina of the Technical University of Cartagena for their help and assistance in lab procedures, as well as Carlos Romero, Ibrahim Tunç, and Javier Ortigosa Castro of the same university for their help in field and lab work.

References

Abou-Elela, S.I., M.M. Kamel, and M.E. Fawzy. 2010. Biological treatment of saline wastewater using a salt-tolerant microorganism. Desalination 250(1): 1–5. doi: 10.1016/j.desal.2009.03.022.

Addy, K., A.J. Gold, L.E. Christianson, M.B. David, L.A. Schipper, et al. 2016. Denitrifying Bioreactors for Nitrate Removal: A Meta-Analysis. J. Environ. Qual. 45(3): 873. doi: 10.2134/jeq2015.07.0399.

Albina, P., N. Durban, A. Bertron, A. Albrecht, J.C. Robinet, et al. 2019. Influence of hydrogen electron donor, alkaline ph, and high nitrate concentrations on microbial denitrification: A review. Int. J. Mol. Sci. 20(20). doi: 10.3390/ijms20205163.

Álvarez-Rogel, J., M. del Carmen Tercero, M.I. Arce, M.J. Delgado, H.M. Conesa, et al. 2016. Nitrate removal and potential soil N2O emissions in eutrophic salt marshes with and without Phragmites australis. Geoderma 282: 49–58. doi: 10.1016/j.geoderma.2016.07.011.

Aparicio, J., L. Candela, O. Alfranca, and J.L. García-Aróstegui. 2017. Economic evaluation of small desalination plants from brackish aquifers. Application to Campo de Cartagena (SE Spain). Desalination 411: 38–44. doi: 10.1016/j.desal.2017.02.004.

Beliavski, M., I. Meerovich, S. Tarre, and M. Green. 2010. Biological denitrification of brines from membrane treatment processes using an upflow sludge blanket (USB) reactor. Water Sci. Technol. 61(4): 911–917. doi: 10.2166/wst.2010.613.

Bell, N., R.A.C. Cooke, T. Olsen, M.B. David, and R. Hudson. 2015. Characterizing the Performance of Denitrifying Bioreactors during Simulated Subsurface Drainage Events. J. Environ. Qual. 44(5): 1647. doi: 10.2134/jeq2014.04.0162.

Bosko, M.L., M.A.S. Rodrigues, J.Z. Ferreira, E.E. Miró, and A.M. Bernardes. 2014. Nitrate reduction of brines from water desalination plants by membrane electrolysis. J. Memb. Sci. 451: 276–284. doi: 10.1016/j.memsci.2013.10.004.

Brettar, I., J.-M. Sanchez-Perez, and M. Tremolieres. 2002. Nitrate elimination by denitrification in hardwood forest soils of the Upper Rhine floodplain- Correlation with redox potential and organic matter. Entomol. Exp. Appl. 469(1–3): 11–21. doi: 10.1023/A:1015527611350.

Bridgham, S.D., K. Updegraff, and J. Pastor. 1998. Carbon, nitrogen, and phosphorus mineralization in northern wetlands. Ecology 79(5): 1545–1561. doi: 10.1890/0012-9658(1998)079[1545:CNAPMI]2.0.CO;2.

Cameron, S.G., and L.A. Schipper. 2010. Nitrate removal and hydraulic performance of organic carbon for use in denitrification beds. Ecol. Eng. 36(11): 1588–1595. doi: 10.1016/j.ecoleng.2010.03.010.

Castejón-Porcel, G., D. Espín-Sánchez, V. Ruiz-Álvarez, R. García-Marín, and D. Moreno-Muñoz. 2018. Runoff water as a resource in the Campo de Cartagena (region of Murcia): Current possibilities for use and benefits. Water 10(4): 1–25. doi: 10.3390/w10040456.

Chow, A.T., K.K. Tanji, S. Gao, and R.A. Dahlgren. 2006. Temperature, water content and wet-dry cycle effects on DOC production and carbon mineralization in agricultural peat soils. Soil Biol. Biochem. 38(3): 477–488. doi: 10.1016/j.soilbio.2005.06.005.

Christianson, L., A. Bhandari, and M. Helmers. 2009. Emerging technology: Denitrification bioreactors for nitrate reduction in agricultural waters. J. Soil Water Conserv. 64(5): 139–141. doi: 10.2489/jswc.64.5.139A.

Christianson, L., A. Castelló, R. Christianson, M. Helmers, and A. Bhandari. 2010. Hydraulic property determination of denitrifying bioreactor fill mediaz. Appl. Eng. Agric. 26(5): 849–854. doi: 10.13031/2013.34946.

Christianson, L., Cooke, R., C. Hay, Helmers, M., G. Feyereisen, et al. 2021. Effectiveness of denitrifying bioreactors on water pollutant reduction from agricultural areas. Am. Soc. Agric. Biol. Eng. doi: 10.13031/trans.14011.

Christianson, L., G. Feyereisen, C. Hay, U. Tschirner, K. Kult, et al. 2020. Denitrifying Bioreactor Woodchip Recharge: Media Properties After Nine Years. ASABE Res. 63(2): 407–416. doi: https://doi.org/10.13031/trans.13709 407.

Christianson, L.E., and M. Helmers. 2011. Woodchip Bioreactors for Nitrate in Agricultural Drainage. Iowa State Univ. Ext. Publ. PMR 1008.: 1–4. http://www.leopold.iastate.edu/sites/default/files/pubs-and-papers/2011-11-woodchip-bioreactors-nitrate-agricultural-drainage.pdf.

Christianson, L., C. Lepine, S. Tsukuda, K. Saito, and S. Summerfelt. 2015. Nitrate removal effectiveness of fluidized sulfur-based autotrophic denitrification biofilters for recirculating aquaculture systems. Aquac. Eng. 68: 10–18. doi: 10.1016/j.aquaeng.2015.07.002.

Christianson, L., and J.C. Tyndall. 2011. Seeking a dialogue: A targeted technology for sustainable agricultural systems in the American Corn Belt. Sustain. Sci. Pract. Policy 7(2): 70–77. doi: 10.1080/15487733.2011.11908075.

Cooke, R.A., A.M. Doheny, and M.C. Hirschi. 2001. Bio-reactors for edge-of-field treatment of tile outflow. ASABE Meet. Present. 01–2018: 1–17. doi: 10.13031/2013.7373.

Craft, C. 2007. Freshwater input structures soil properties , vertical accretion , and nutrient accumulation of Georgia and U . S . tidal marshes. Limnol. Oceanogr. Methods 52(3): 1220–1230.

Díaz-García, C., J.J. Martínez-Sánchez, and J. Álvarez-Rogel. 2020. Bioreactors for brine denitrification produced during polluted groundwater desalination in fertigation areas of SE Spain : batch assays for substrate selection. Environ. Sci. Pollut. Res. (2): 1–10. doi: https://doi.org/10.1007/s11356-020-09567-6.

Dinçer, A.R., and F. Kargi. 2000. Effects of operating parameters on performances of nitrification and denitrification processes. Bioprocess Eng. 23(1): 75–80. doi: 10.1007/s004499900126.

Donnelly, K. 2014. The Red Sea-Dead sea project update. The World's Water. p. 153–158

Ersever, I., V. Ravindran, and M. Pirbazari. 2007. Biological denitrification of reverse osmosis brine concentrates: II. Fluidized bed adsorber reactor studies. J. Environ. Eng. Sci. 6(5): 519–532. doi: 10.1139/S07-009.

FAO. 2017. FAOSTAT. http://www.fao.org/faostat/en/#data/QC (accessed 26 February 2019).

Feyereisen, G.W., T.B. Moorman, L.E. Christianson, R.T. Venterea, J.A. Coulter, et al. 2016. Performance of Agricultural Residue Media in Laboratory Denitrifying Bioreactors at Low Temperatures. J. Environ. Qual. 45(3): 779. doi: 10.2134/jeq2015.07.0407.

Fiedler, S., M.J. Vepraskas, and J.L. Richardson. 2007. Soil Redox Potential: Importance, Field Measurements, and Observations. Adv. Agron. 94(06): 1–54. doi: 10.1016/S0065-2113(06)94001-2.

Greenan, C.M., T.B. Moorman, T.B. Parkin, T.C. Kaspar, and D.B. Jaynes. 2009. Denitrification in Wood Chip Bioreactors at Different Water Flows. J. Environ. Qual. 38(4): 1664. doi: 10.2134/jeq2008.0413.

Grießmeier, V., and J. Gescher. 2018. Influence of the potential carbon sources for field denitrification beds on their microbial diversity and the fate of carbon and nitrate. Front. Microbiol. 9(JUN): 1–12. doi: 10.3389/fmicb.2018.01313.

Halaburka, B.J., G.H. Lefevre, and R.G. Luthy. 2017. Evaluation of Mechanistic Models for Nitrate Removal in Woodchip Bioreactors. Environ. Sci. Technol. 51(9): 5156–5164. doi: 10.1021/acs.est.7b01025.

Hansson, K., D.B. Kleja, K. Kalbitz, and H. Larsson. 2010. Amounts of carbon mineralised and leached as DOC during decomposition of Norway spruce needles and fine roots. Soil Biol. Biochem. 42(2): 178–185. doi: 10.1016/j.soilbio.2009.10.013.

Healy, M.G., T.G. Ibrahim, G.J. Lanigan, A.J. Serrenho, and O. Fenton. 2012. Nitrate removal rate, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors. Ecol. Eng. 40: 198–209. doi: 10.1016/j.ecoleng.2011.12.010.

Hoegh-Guldberg, O., D. Jacob, and M. Taylor. 2018. 2018: Impacts of 1.5^{o}C Global Warming on Natural and Human Systems. In: Global Warming of 1.5°C. 2018 Impacts 1.5^{o}C Glob. Warm. Nat. Hum. Syst. Glob. Warm. 1.5°C: 175–311. https://www.ipcc.ch/sr15.

Hoover, N.L., A. Bhandari, M.L. Soupir, and T.B. Moorman. 2016. Woodchip Denitrification Bioreactors: Impact of Temperature and Hydraulic Retention Time on Nitrate Removal. J. Environ. Qual. 45(3): 803–812. doi: 10.2134/jeq2015.03.0161.

Howarth, R.W. 2008. Coastal nitrogen pollution: A review of sources and trends globally and regionally. Harmful Algae 8(1): 14–20. doi: 10.1016/j.hal.2008.08.015.

IDALS. 2014. Iowa Nutrient Reduction Strategy: A science and technology-based framework to assess and reduce nutrients to Iowa waters and the Gulf of Mexico. http://www.nutrientstrategy.iastate.edu/ (accessed 15 November 2020).

IPCC. 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability (C. Field and V. Barros, editors). Cambrisge University Press, Cambridge and New York.

Jiménez-Martínez, J., R. Aravena, and L. Candela. 2011. The role of leaky boreholes in the contamination of a regional confined aquifer. A case study: The campo de cartagena region, Spain. Water. Air. Soil Pollut. 215(1–4): 311–327. doi: 10.1007/s11270-010-

0480-3.

Jiménez-Martínez, J., J.L. García-Aróstegui, J.E. Hunink, S. Contreras, P. Baudron, et al. 2016. The role of groundwater in highly human-modified hydrosystems: A review of impacts and mitigation options in the Campo de Cartagena-Mar Menor coastal plain (SE Spain). Environ. Rev. 24(4): 377–392. doi: 10.1139/er-2015-0089.

Lay, W.C.L., Y. Liu, and A.G. Fane. 2010. Impacts of salinity on the performance of high retention membrane bioreactors for water reclamation: A review. Water Res. 44(1): 21–40. doi: 10.1016/j.watres.2009.09.026.

Lewis, W.M., W.A. Wurtsbaugh, and H.W. Paerl. 2011. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. Environ. Sci. Technol. 45(24): 10300–10305. doi: 10.1021/es202401p.

Lysbakken, K.R. 2013. Salting of Winter Roads : The Quantity of Salt on Road Surfaces after Application. (August). http://www.diva-portal.org/smash/get/diva2:660746/FULLTEXT02.pdf.

Manca, F., D. De Rosa, L.P. Reading, D.W. Rowlings, C. Scheer, et al. 2020. Nitrate removal and greenhouse gas production of woodchip denitrification walls under a humid subtropical climate. Ecol. Eng. 156(April): 105988. doi: 10.1016/j.ecoleng.2020.105988.

Marton, J.M., E.R. Herbert, and C.B. Craft. 2012. Effects of salinity on denitrification and greenhouse gas production from laboratory-incubated tidal forest soils. Wetlands 32(2): 347–357. doi: 10.1007/s13157-012-0270-3.

Masbough, A., K. Frankowski, K.J. Hall, and S.J.B. Duff. 2005. The effectiveness of constructed wetland for treatment of woodwaste leachate. Ecol. Eng. 25(5): 552–566. doi: 10.1016/j.ecoleng.2005.07.006.

Maxwell, B.M., F. Birgand, L.A. Schipper, L.E. Christianson, S. Tian, et al. 2018. Drying–Rewetting Cycles Affect Nitrate Removal Rates in Woodchip Bioreactors. J. Environ. Qual. 48(1): 93–101. doi: 10.2134/jeq2018.05.0199.

Maxwell, B.M., C. Díaz-García, J.J. Martínez-Sánchez, and J. Álvarez-Rogel. 2020a. Temperature sensitivity of nitrate removal in woodchip bioreactors increases with woodchip age and following drying – rewetting cycles. Environ. Sci. Water Technol. (3): 3–5. doi: 10.1039/d0ew00507j.

Maxwell, B., C. Díaz-García, J.J. Martínez-Sánchez, and J. Álvarez-Rogel. 2020b. Increased brine concentration increases nitrate reduction rates in batch woodchip bioreactors treating brine from desalination. Desalination 495: 114629. doi: 10.1016/j.desal.2020.114629.

Ministerio de Agricultura Alimentación y Medio Ambiente. 2013. Guía Metodológica.

Ministry for Ecological Transition of Spain. 2020. Estudio sobre el estado de la Mar Menor. Campo de Cartagena a los efectos de la procedencia de su declaración de en riesgo de no alcanzar el buen estado cuantitativo o químico.

Moorman, T.B., T.B. Parkin, T.C. Kaspar, and D.B. Jaynes. 2010. Denitrification activity, wood loss, and N2O emissions over 9 years from a wood chip bioreactor. Ecol. Eng. 36(11): 1567–1574. doi: 10.1016/j.ecoleng.2010.03.012.

Murcia Regional Government. 2018. Ley 1/2018, de 7 de febrero, de medidas urgentes para garantizar la sostenibilidad ambiental en el entorno del Mar Menor. Boletín Oficial de la Región de Murcia, Spain.

Murcia Regional Government. 2020. Ley 3/2020, de 27 de julio, de recuperación y protección del Mar Menor. Spain.

Otero, X.L., and F. Macias. 2003. Spatial variation in pyritization of trace metals in salt-marsh soils. Biogeochemistry 62(1): 59–86. doi: 10.1023/A:1021115211165.

Palomar, P., and I.J. Losada. 2010. Desalination in Spain: Recent developments and recommendations. Desalination 255(1–3): 97–106. doi: 10.1016/j.desal.2010.01.008.

Ponnamperuma, F.N., E. Martinez, and T. Loy. 1966. Influence of redox potential and partial pressure of carbon dioxide on pH values and the suspension effect of flooded soils. Soil Sci. 101(6): 421–431.

Povilaitis, A., A. Rudzianskaitė, S. Misevičienė, V. Gasiūnas, O. Miseckaitė, et al. 2018. Efficiency of Drainage practices for improving water quality in Lithuania. Am. Soc. Agric. Biol. Eng. 61(1): 179–196. doi: https://doi.org/10.13031/trans.12271 179.

Reddy, K.R.R., and R.D. DeLaune. 2008. Biogeochemistry of Wetlands. CRC Press, Boca Raton.

Rivett, M.O., S.R. Buss, P. Morgan, J.W.N. Smith, and C.D. Bemment. 2008. Nitrate attenuation in groundwater: A review of biogeochemical controlling processes. Water Res. 42(16): 4215–4232. doi: 10.1016/j.watres.2008.07.020.

Robertson, W.D. 2010. Nitrate removal rates in woodchip media of varying age. Ecol. Eng. 36(11): 1581–1587. doi: 10.1016/j.ecoleng.2010.01.008.

Robertson, W.., and D.. Blowes. 2000. Long-Term Performance of In Situ Reactive Barriers for Nitraye Remediation. Ground Water 38(5): 689–695. https://search-proquest-com.uri.idm.oclc.org/docview/236850512/fulltextPDF/1276377CB22D46E2PQ/1?accountid=28991.

Robertson, W.D., and L.C. Merkley. 2009. In-Stream Bioreactor for Agricultural Nitrate Treatment. J. Environ. Qual. 38(1): 230. doi: 10.2134/jeq2008.0100.

RStudio. 2020. RStudio: Integrated Development for R. https://rstudio.com/ (accessed 3 December 2020).

Ruiz-Fernández, J., V. León, L. Marín-Guirao, F. Giménez-Casaduero, J. Rogel, et al. 2019. Synthesis report of the current state of Mar Menor lagoon and its causes in relation to the nutrient contents.

Rysgaard, Sø., P. Thastum, T. Dalsgaard, P.B. Christensen, and N.P. Sloth. 1999. Effects of salinity on NH4+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. Estuaries 22(1): 21–30. doi: 10.2307/1352923.

Saliling, W.J.B., P.W. Westerman, and T.M. Losordo. 2007. Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. Aquac. Eng. 37(3): 222–233. doi: 10.1016/j.aquaeng.2007.06.003.

Sánchez, A.S., I.B.R. Nogueira, and R.A. Kalid. 2015. Uses of the reject brine from inland desalination for fish farming, Spirulina cultivation, and irrigation of forage shrub and crops. Desalination 364: 96–107. doi: 10.1016/j.desal.2015.01.034.

Schipper, L.A., G.F. Barkle, and M. Vojvodic-Vukovic. 2005. Maximum Rates of Nitrate Removal in a Denitrification Wall. J. Environ. Qual. 34(4): 1270–1276. doi: 10.2134/jeq2005.0008.

Schipper, L.A., W.D. Robertson, A.J. Gold, D.B. Jaynes, and S.C. Cameron. 2010. Denitrifying bioreactors-An approach for reducing nitrate loads to receiving waters. Ecol. Eng. 36(11): 1532–1543. doi: 10.1016/j.ecoleng.2010.04.008.

Schipper, L.A., and M. Vojvodić-Vuković. 2001. Five years of nitrate removal, denitrification and carbon dynamics in a denitrification wall. Water Res. 35(14): 3473–3477. doi: 10.1016/S0043-1354(01)00052-5.

Scientific Advisory Group for El Mar Menor. 2017. Informe integral sobre el estado ecológico del Mar Menor.

SPSS. 2010. SPSS 19.0 for Windows software IBM Company.

Steele, M.K., and J.A. Aitkenhead-Peterson. 2013. Salt impacts on organic carbon and nitrogen leaching from senesced vegetation. Biogeochemistry 112(1–3): 245–259. doi: 10.1007/s10533-012-9722-3.

Stumm, W., and B. Sulzberger. 1992. The cycling of iron in natural environments: Considerations based on laboratory studies of heterogeneous redox processes. Geochim. Cosmochim. Acta 56(8): 3233–3257. doi: 10.1016/0016-7037(92)90301-X.

Sukias, J.P.S., J.B.K. Park, R. Stott, and C.C. Tanner. 2018. Quantifying treatment system resilience to shock loadings in constructed wetlands and denitrification bioreactors. Water Res. 139: 450–461. doi: 10.1016/j.watres.2018.04.010.

Tercero, M.C., J. Álvarez-Rogel, H.M. Conesa, M.A. Ferrer, A.A. Calderón, et al. 2015. Response of biogeochemical processes of the water-soil-plant system to experimental flooding-drying conditions in a eutrophic wetland: the role of Phragmites australis. Plant Soil 396(1–2): 109–125. doi: 10.1007/s11104-015-2589-z.

Tragsatec. 2020. Modelo de Flujo. Acuífero Cuaternario del Campo de Cartagena: Cuantificación, Control de Calidad y Seguimiento Piezométrico de la Descarga de Agua Subterránea del Acuífero Cuaternario del Campo de Cartagena al Mar Menor.

Trögl, J., A. Boušková, J. Mrákota, V. Pilařová, J. Krudencová, et al. 2011. Removal of nitrates from simulated ion-exchange brines with Paracoccus denitrificans encapsulated in Lentikats Biocatalyst. Desalination 275(1–3): 82–86. doi:

10.1016/j.desal.2011.02.033.

Trögl, J., O. Krhůtková, V. Pilařová, P. Dáňová, R. Holíček, et al. 2012. Removal of nitrates from high-salinity wastewaters from desulphurization process with denitrifying bacteria encapsulated in Lentikats Biocatalyst. Int. J. Environ. Sci. Technol. 9(3): 425–432. doi: 10.1007/s13762-012-0048-4.

USDA. 2015. Conservation Practice Standard 605: Denitrifying bioreactor. Washington, DC.

Vepraskas, M.J., and S.P. Faulker. 2001. Chapter 4: Redox Chemistry of Hydric Soils.pdf. Wetlands soils: Genesis, Hydrology, Landscape and Classification. Lewis Publishers. p. 85–105

von Ahnen, M., S.L. Aalto, S. Suurnäkki, M. Tiirola, and P.B. Pedersen. 2019. Salinity affects nitrate removal and microbial composition of denitrifying woodchip bioreactors treating recirculating aquaculture system effluents. Aquaculture: 22. doi: 10.1016/j.aquaculture.2019.01.068.

von Ahnen, M., P.B. Pedersen, and J. Dalsgaard. 2016a. Start-up performance of a woodchip bioreactor operated end-of-pipe at a commercial fish farm—A case study. Aquac. Eng. 74: 96–104. doi: 10.1016/j.aquaeng.2016.07.002.

von Ahnen, M., P.B. Pedersen, C.C. Hoffmann, and J. Dalsgaard. 2016b. Optimizing nitrate removal in woodchip beds treating aquaculture effluents. Aquaculture 458: 47–54. doi: 10.1016/j.aquaculture.2016.02.029.

Vymazal, J. 2014. Constructed wetlands for treatment of industrial wastewaters: A review. Ecol. Eng. 73: 724–751. doi: 10.1016/j.ecoleng.2014.09.034.

Wang, J., and L. Chu. 2016. Biological nitrate removal from water and wastewater by solid-phase denitrification process. Biotechnol. Adv. 34(6): 1103–1112. doi:

10.1016/j.biotechadv.2016.07.001.

Warneke, S., L.A. Schipper, D.A. Bruesewitz, I. McDonald, and S. Cameron. 2011. Rates, controls and potential adverse effects of nitrate removal in a denitrification bed. Ecol. Eng. 37(3): 511–522. doi: 10.1016/j.ecoleng.2010.12.006.

Weston, N.B., M.A. Vile, S.C. Neubauer, and D.J. Velinsky. 2011. Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils. Biogeochemistry (102): 135–151. doi: 10.1007/s10533-010-9427-4.

Wisniewski, C., F. Persin, T. Cherif, R. Sandeaux, A. Grasmick, et al. 2002. Use of a membrane bioreactor for denitrification of brine from an electrodialysis process. Desalination 149(1–3): 331–336. doi: 10.1016/S0011-9164(02)00805-6.

Xu, X., Y. Luo, and J. Zhou. 2012. Carbon quality and the temperature sensitivity of soil organic carbon decomposition in a tallgrass prairie. Soil Biol. Biochem. 50: 142–148. doi: 10.1016/j.soilbio.2012.03.007.

Xu, X., J. Zhu, J.E. Thies, and W. Wu. 2020. Methanol-linked synergy between aerobic methanotrophs and denitrifiers enhanced nitrate removal efficiency in a membrane biofilm reactor under a low O2:CH4 ratio. Water Res. 174: 115595. doi: 10.1016/j.watres.2020.115595.