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Evaluation of passive reduction of nitrate from greenhouse effluent by planted bioreactors

S. Fatehi Pouladi, B. C. Anderson, B. Wootton and L. Rozema

ABSTRACT

A number of pilot-scale gravel and wood-chip hybrid bioreactors planted with select species, together with unplanted units, were evaluated for their nutrient removal capabilities from the typical greenhouse effluent with high levels of nitrate and salts. Two levels of nutrient solution (high and low loading: HL/LL) were prepared to simulate the typical characteristics of the greenhouse effluent. The wood-chip bioreactor with *Typha angustifolia* exhibited the highest consistent nutrient treatment with an average nitrate reduction in the LL phase of 88.4% (28.2 g N m^{-3} media day^{-1}) and phosphate reduction of 34.4%. The nitrate reduction in this bioreactor was the highest among the values reported in the literature. The near-complete denitrification developed provided a nitrate-limiting environment as evidenced by an average 21.5% sulfate reduction. The distinct increase in the outflow organic carbon (as BOD_5) from the wood chips in the bioreactor planted with *T. angustifolia* appeared to be the key explanation for the efficient denitrification, while the other vegetated bioreactors resulted in 19.0%–36.5% nitrate reduction and low outflow BOD_5 near the end of the experiment, indicating carbon limitation in these bioreactors.

Key words | denitrification, greenhouse effluent, nitrate, wood-chip bioreactor

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INTRODUCTION

Indoor crop production in greenhouses is recognized as an efficient, year-round farming practice, mainly for crop yield and controlled use of resources. The typical effluent generated by greenhouses for food production contains high levels of nitrate, among other problematic constituents (Saxena & Bassi 2013). Although the greenhouse effluent is typically low in organic carbon concentrations (Prystay & Lo 2001), the direct discharge of this nutrient-rich solution to the surrounding surface waters has major environmental risks including eutrophication and hypoxia (Gruyer *et al.* 2013). Recently in Ontario, Canada, new regulations came into effect to help greenhouse growers comply with environmental standards for managing the effluent from closed-system operations (GNF 2015). Furthermore, the health implications caused by the high nitrate level in drinking water have resulted in the enforcement of a maximum acceptable concentration for nitrate in drinking water (10 mg $NO_3^- - N$ L^{-1}; Health Canada 2013).

Denitrification is commonly recognized as a process in which nitrate is reduced to nitrogen gas via other intermediates. As most of the denitrification is carried out by facultative heterotrophs, the process is strongly dependent on carbon availability (Kadlec & Wallace 2009). Many on-site subsurface systems designed for treating wastewater with high nitrate concentrations and low organic matter employ wood chips to supply the stream with the organic carbon required to facilitate denitrification (e.g. Christianson *et al.* 2012; Ghane *et al.* 2015). While other carbon-rich sources have been studied to supplement these treatment systems, wood wastes are known as the best and most

doi: 10.2166/ws.2017.080

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commonly used materials for denitrification systems (Bednarek *et al.* 2014). A meta-analysis of several studies suggested that wood source did not significantly affect the nitrate removal rates in denitrifying beds (Addy *et al.* 2016), and wood chips in particular were reported elsewhere to provide ideal conditions for denitrifying bacteria (Warneke *et al.* 2011c).

The different aspects of engineered denitrifying treatment systems, known collectively as denitrifying bioreactors, have recently been investigated in the treatment of diffuse agricultural runoff. The increasing number of recent publications, together with the official inclusion of wood-chip bioreactors in the nutrient reduction strategies of some of the US Midwestern states (Christianson & Schipper 2016), show the effective applicability of these systems for nitrate reduction. The majority of nitrate removal in similar systems was reported to occur due to heterotrophic denitrification, while the role of other processes such as dissimilatory nitrate reduction to ammonium, anammox and plant uptake were presumed to be relatively low (Warneke *et al.* 2011a).

Despite the promising results made available in the literature during the past few years, as well as confirmed multi-year longevity and nitrate removal as high as 16.1 g N m^{-3} media day^{-1} for fresh wood chips in test columns (Robertson 2010) and up to 22 g N m^{-3} media day^{-1} in denitrification beds (Schipper *et al.* 2010b), the removal rates remain varied among different studies (Addy *et al.* 2016). In addition, and as mentioned previously, the majority of the published studies on wood-chip bioreactors and their high effectiveness for nitrate management have focused on real or simulated agricultural tile drainage (e.g. Woli *et al.* 2010; Christianson *et al.* 2011; David *et al.* 2016) with NO_3^-–N inflow concentrations typically below 60 mg L^{-1}. The typical greenhouse effluent generated by indoor vegetable and fruit producers, however, contains much higher NO_3^-–N concentrations, usually in the range of 200 to 325 mg L^{-1} (Park *et al.* 2009; Schipper *et al.* 2010a).

The studies on the performance of wood-chip bioreactors subject to greenhouse effluent are scarce and limited to one greenhouse (glasshouse) located in New Zealand (Schipper *et al.* 2010a; Warneke *et al.* 2011b). The denitrification bioreactor (bed) employed at this site resulted in long-term nitrate removal between 5 and 10 g N m^{-3} (about 40% N removal) while the nitrate outflow concentrations were not limiting and remained mostly greater than 100 mg N L^{-1}. The system was regarded as being overwhelmed by the high inflow nitrate concentration and large flow rate (Schipper *et al.* 2010a).

The common design of denitrification beds investigated in the literature consists of underground excavations or trenches filled with organic substrate which receive the nitrogen-rich solution from one end and discharge the treated water from the other end (e.g. Addy *et al.* 2016), resembling the well-known configuration of horizontal-flow constructed wetlands (CWs). However, the performance and efficacy of denitrifying bioreactors designed to operate in vertical-flow mode has not been assessed. Most importantly, the low-cost denitrification bioreactors are usually not vegetated due to their specific construction layout under the ground which aims to provide the anaerobic conditions necessary for a successful denitrification process. The positive roles of vegetation in removing nutrients in vertical-flow CWs (e.g. Kantawanichkul *et al.* 2009) and in wood-chip bioreactors for salt removal (Fatehi Pouladi *et al.* 2016) have been studied. However, the potential contributions of vegetation to nutrient removal in hybrid wood-chip bioreactors have not been discussed. As such, it is not clear how the presence of different plant species in hybrid wood-chip bioreactors would influence the microbial activity and available organic matter that are generally considered responsible for the efficacy of the systems by facilitating denitrification. Additionally, it has been suggested that those wetland plants found in the area of a wetland be considered for use in CWs for agricultural wastewater, with *Typha* as the most common species used in northeastern North America (Rozema *et al.* 2016).

The objectives of the present study were to investigate the efficiency of the vertical-flow bioreactors in treating greenhouse effluent, and to compare the influence of multiple plant species on nutrient reduction, with particular focus on inorganic nitrate. The main hypothesis for which the experiments were designed was that hybrid denitrification systems equipped with phytotechnology would enhance the overall treatment performance, primarily due to the potential enhanced biological contributions provided by the plants.

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MATERIALS AND METHODS

This study consisted of two bioreactor experiments with gravel and wood chips as the media, the phytodesalination performance of which was reported in Fatehi Pouladi *et al.* (2016). The gravel pilot-scale experiment was conducted at the Centre for Alternative Wastewater Treatment (CAWT) in Lindsay, Ontario, Canada, and the wood-chip bioreactors were operated at the laboratory of the Department of Civil Engineering at Queen's University in Kingston, Ontario, Canada. The main objective of the gravel experiment was to evaluate nutrient reduction by the planted bioreactors in the absence of an organic-rich substrate, while the wood-chip bioreactors were designed to assess the changes in water quality parameters of the greenhouse effluent by supplying organic carbon from the wood chips in conjunction with the presence of established vegetation.

The general layout, operation and treatment mechanism in our gravel and wood-chip bioreactors were similar to the vertical-flow subsurface CWs. Each reactor was built using a 220 L open-top barrel (56 cm in diameter, 90 cm in height) filled with a single 80 cm layer of the media substrate. The influent used to feed the bioreactors was synthesized in the laboratory to mimic the typical characteristics of the greenhouse effluent. One perforated PVC grid on the surface of the media distributed the influent evenly over each reactor's surface, and another duplicate grid was placed at the bottom of the barrel to collect the treated water and direct it to the outlet pipe for discharge and sample collection. The outlet pipe created saturated conditions with the reactors operating in vertical down-flow (top–bottom) mode providing low levels of dissolved oxygen within the unit. The synthetic influent (continuous flow rate: 30 L day^{-1}) was made using commercial fertilizer, NaCl and Na_2SO_4 in water. The hydraulic residence time (HRT) was approximately 3.3 days for the gravel bioreactors (assumed gravel porosity: 0.5) and 3.7 days for the wood-chip bioreactors with the measured porosity of 0.58 (Fatehi Pouladi *et al.* 2016).

The five vegetated gravel reactors together with one unplanted (control) unit were housed in a greenhouse with natural sunlight and an ambient temperature range of 15 to 25 °C. The substrate media was 9.5 mm (3/8 in) gravel with no sand or fines. In the wood-chip bioreactors, one unplanted unit and a maximum of four vegetated reactors were operated under a metal halide grow light set to a continuous 16/8 hour on/off cycle per 24 hours (average daytime ambient temperature: 23.6 °C). The wood-chip media was composed of 2–3 cm long grains and was sourced from an agricultural facility in Quebec, Canada. The planted bioreactors in both experiments contained approximately similar cover density for various plants, with about 7 to 8 plants per barrel and 30 plants m^{-2} in cover density. Two levels of nutrient loading were created for the wood-chip bioreactors' influent (Table 1). This was done to account for the low (low loading: LL) and high (high loading: HL) ends of nitrate concentration ranges in the typical greenhouse effluent. A total of seven plant species were tested in the experiments including softstem bulrush (*Schoenoplectus tabernaemontani* C.C. Gmel. Palla, abbreviated as

Table 1 | The active plant species used in each experimental period

		Operation period		Operated reactors							
Reactor type	**Phase**	**From**	**To**	***S. taber.***	***A. gerardii***	***T. angustifolia***	***E. canadensis***	***P. virgatum***	***S. pectinata***	***D. spicata***	**Control**
Gravel	–	12 Mar 2014	02 Jul 2014	■	■	■	■	■	–	–	■
Wood-chip HL[a]	Phase 1	01 Oct 2014	07 Apr 2015	■	–	■	■	■	–	–	■
	Interim	08 Apr 2015	11 Jun 2015	■	–	■	–	–	–	–	■
	Phase 2	12 Jun 2015	23 Oct 2015	■	–	■	–	–	■	■	■
Wood-chip LL[b]	–	24 Oct 2015	17 May 2016	■	–	■	–	–	■	■	■

Highlighted cells represent the reactors in use.
[a]High loading.
[b]Low loading.

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S. taber. in the following tables), big bluestem (*Andropogon gerardii* Vitman), narrowleaf cattail (*Typha angustifolia* L.), Canada wildrye (*Elymus canadensis* L.), switchgrass (*Panicum virgatum* L.), prairie cordgrass (*Spartina pectinata* Bosc ex Link) and saltgrass (*Distichlis spicata* L. Greene). Table 1 summarizes the active species for each experiment and their timelines.

Grab samples were collected from the influent and the discharged solution from the bioreactors for water quality analysis according to *Standard Methods* (APHA 2005). The gravel experiment data for the first week of operation were omitted as the values were considered unusually low. The change in concentration was calculated as

$$R = \frac{100 \times (\Delta C)}{C_{\mathrm{i}}}$$

where R (%) is the percentage removal of the target constituent, and ΔC ($\mathrm{mg\,L^{-1}}$) is the difference between the constituent's averaged concentration in the inflow (C_{i}, $\mathrm{mg\,L^{-1}}$) and that in the outflow after treatment (C_{e}, $\mathrm{mg\,L^{-1}}$) at the point of discharge. As the effect of evapotranspiration on concentration change was demonstrated to be relatively small (1.9%, Fatehi Pouladi *et al.* 2016), a mass balance for a target constituent inside the reactor can be defined as

$$\frac{\Delta M}{\Delta t} = Q \times \Delta C$$

where $\Delta M/\Delta t$ ($\mathrm{g\,day^{-1}}$) is the mass removal rate of the target constituent, Q ($\mathrm{L\,day^{-1}}$) is the incoming flow rate and ΔC is in $\mathrm{g\,L^{-1}}$. The nitrate mass removal rate was defined according to

$$dm_{\mathrm{NO_3^-{-}N}} = \frac{Q \times \Delta C}{V_{\mathrm{media}}}$$

where $dm_{\mathrm{NO_3^-{-}N}}$ ($\mathrm{g\,day^{-1}\,m^{-3}}$) is the nitrate mass removal rate, V_{media} ($\mathrm{m^3}$) is the volume of the bioreactor filled with wood-chip media and ΔC is in $\mathrm{g\,L^{-1}}$. The nitrate removal rates were defined as

$$r_{\mathrm{NO_3^-{-}N}} = \frac{\Delta C}{HRT}, \text{ and}$$

$$k_{\mathrm{NO_3^-{-}N}} = \frac{Ln(C_{\mathrm{i}}/C_{\mathrm{e}})}{HRT}$$

where $r_{\mathrm{NO_3^-{-}N}}$ ($\mathrm{mg\,L^{-1}\,day^{-1}}$) is the average zero-order nitrate removal rate over the operation period of each experiment, ΔC is in $\mathrm{mg\,L^{-1}}$, HRT (day) is the estimated hydraulic residence time of each wood-chip bioreactor (3.7 days) and $k_{\mathrm{NO_3^-{-}N}}$ ($\mathrm{day^{-1}}$) is the average first-order nitrate removal rate.

Using XLStat software (©Addinsoft), one-way analysis of variance (ANOVA), Tukey's and Dunnett's methods were employed for statistical analysis in order to identify significant differences between the reactors (difference reported significant when *p-value* <0.05). The water quality sampling from the wood-chip bioreactors was delayed for 70 and 27 days in Phase 1 and Phase 2 of the HL experiment respectively to allow for acclimation within the bioreactors, whereas the LL component started immediately after Phase 2 as the bioreactors had been in operation for over 1 year.

RESULTS AND DISCUSSION

The planted gravel bioreactors did not result in any significantly better performance compared with the control gravel unit (Table 2, Figure 1). The overall zero nitrate

Table 2 | Average parameters of the inflow (influent) and outflow solutions in gravel bioreactors

Parameter (unit)	Influent		Control	Planted Reactors
$\mathrm{NO_3^-{-}N}$ ($\mathrm{mg\,L^{-1}}$)	204.1 [28.4]	Out[a]	204.5 [25.6]	210.4 [4.2]
		Red.[b]	–	–
$\mathrm{NO_2^-{-}N}$ ($\mathrm{mg\,L^{-1}}$)	0.2 [0.3]	Out[a]	5.2 [3.1]	4.2 [0.9]
		Red.[b]	–	–
$\mathrm{NH_3{-}N}$ ($\mathrm{mg\,L^{-1}}$)	98.9 [9.2]	Out[a]	87.5 [10.2]	86.7 [1.8]
		Red.[b]	11.5%	12.3%
TKN – N ($\mathrm{mg\,L^{-1}}$)	116.2 [0.9]	Out[a]	98.7 [4.4]	101.4 [2.9]
		Red.[b]	15.1%	12.7%
$\mathrm{PO_4^{3-}{-}P}$ ($\mathrm{mg\,L^{-1}}$)	43.9 [4.0]	Out[a]	36.8 [3.4]	37.6 [0.5]
		Red.[b]	16.2%	14.4%
$\mathrm{SO_4^{2-}{-}S}$ ($\mathrm{mg\,L^{-1}}$)	124.4 [6.0]	Out[a]	124.2 [8.2]	127.8 [3.4]
		Red.[b]	0.2%	–
$\mathrm{BOD_5}$ ($\mathrm{mg\,L^{-1}}$)	2.0 [1.1]	Out[a]	5.4 [4.5]	11.1 [3.3]
COD ($\mathrm{mg\,L^{-1}}$)	34.7 [4.6]		33.6 [1.3]	33.6 [1.8]
pH	7.3 [0.4]		7.2 [0.2]	7.2 [0.1]

SD values are given in brackets.
[a]The measured constituent in the outflow solution.
[b]Reduction with respect to the influent.

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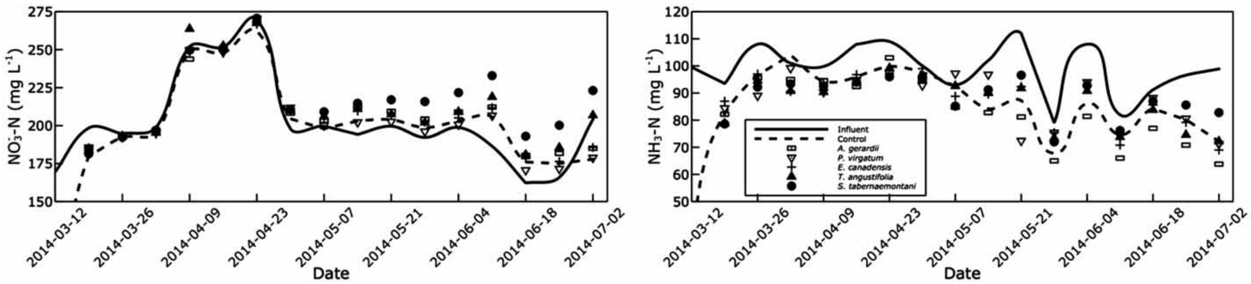

Figure 1 | Temporal trends of nutrient concentrations in gravel reactors (left: $NO_3^- - N$; right: $NH_3 - N$).

removal in these units was expected as the gravel substrate did not provide the organic carbon required to facilitate microbial denitrification, despite the average BOD_5 level being about two times higher in the planted units.

In the HL period of the wood-chip experiment, the $NO_3^- - N$ concentrations in the outflow of the *T. angustifolia* and *S. tabernaemontani* wood-chip units were significantly different from the unplanted bioreactor (*p-value* <0.05, Table 3, Figure 2(a)), demonstrating average reductions of 38.6% and 55.3% respectively. The *S. tabernaemontani* bioreactor, however, showed consistently higher $NO_3^- - N$ outflow concentrations as the experiment continued in time until the end of the high-loading phase. The bioreactors in this phase showed minimal $PO_4^{3-} - P$ reductions (max. 9.2%), while *T. angustifolia* exhibited lower outflow concentrations as the plant became better established in the summer of 2015 (Figure 2(b)).

As the operation of the wood-chip bioreactors transitioned into the next phase, treating low-load influent, the *T. angustifolia* bioreactor was the only vegetated unit that demonstrated significantly different results for most of the

Table 3 | Average parameters of the inflow (influent) and outflow solutions in wood-chip bioreactors receiving the synthetic greenhouse HL effluent

Parameter (unit)	Influent		Control	*E. canadensis*	*P. virgatum*	*S. taber.*	*S. pectinata*	*D. spicata*	*T. angustifolia*
$NO_3^- - N$ (mg L^{-1})	307.3 [9.9]	Out[a]	209.6 [73.8]	172.8 [31.4]	207.4 [20.5]	137.4 [73.0]*	202.9 [33.3]	214.4 [5.2]	188.7 [33.1]*
		Red.[b]	31.8%	43.8%	32.5%	55.3%	34.0%	30.2%	38.6%
$NO_2^- - N$ (mg L^{-1})	1.8 [0.6]	Out[a]	3.2 [0.9]	6.3 [2.7]	2.6 [0.5]	2.1 [0.8]	1.8 [0.8]	2.0 [0.7]	4.9 [4.9]
		Red.[b]	–	–	–	–	–	–	–
$NH_3 - N$ (mg L^{-1})	8.0 [0.8]	Out[a]	6.4 [2.6]	5.1 [1.1]	5.8 [1.3]	8.1 [5.0]	3.1 [3.1]	8.6 [4.3]	8.4 [4.9]
		Red.[b]	20.0%	36.3%	27.5%	–	61.3%	–	–
TKN – N (mg L^{-1})	9.3 [1.8]	Out[a]	8.2 [1.6]	8.5 [1.2]	8.6 [0.7]	12.3 [3.8]	5.0 [2.9]	11.3 [2.9]	10.9 [4.8]
		Red.[b]	11.8%	8.6%	7.5%	–	46.2%	–	–
$PO_4^{3-} - P$ (mg L^{-1})	26.2 [3.5]	Out[a]	25.7 [3.3]	24.3 [1.6]	24.9 [2.4]	25.1 [3.3]	23.8 [0.5]	25.7 [0.5]	24.4 [4.2]
		Red.[b]	1.9%	7.3%	5.0%	4.2%	9.2%	1.9%	6.9%
$SO_4^{2-} - S$ (mg L^{-1})	131.0 [4.1]	Out[a]	132.4 [4.0]	131.7 [2.3]	132.7 [1.1]	130.4 [2.9]	134.4 [2.7]	130.6 [3.2]	136.2 [4.4]
		Red.[b]	–	–	–	0.5%	–	0.3%	–
Alk.[c] (mg L^{-1})	8.6 [3.9]	Out[a]	334.3 [241.3]	428.3 [80.2]	341.4 [56.2]	581.1 [237.7]	349.8 [125.5]	323.5 [41.2]	427.7 [94.6]
BOD_5 (mg L^{-1})	2.8 [1.3]		12.1 [4.6]	11.0 [4.4]	16.0 [6.4]	15.7 [8.9]	9.5 [7.7]	13.8 [3.3]	14.4 [6.4]
COD (mg L^{-1})	32.6 [11.0]		83.5 [65.7]	80.5 [28.3]	110.3 [59.7]	130.0 [54.0]	50.5 [20.6]	70.9 [8.1]	75.9 [22.7]
pH	5.5 [0.5]		7.4 [0.2]	7.3 [0.2]	7.3 [0.2]	7.2 [0.2]	7.3 [0.1]	7.4 [0.1]	7.3 [0.2]

SD values are given in brackets.

[a]The measured constituent in the outflow solution.

[b]Reduction with respect to the influent.

[c]Alkalinity as $CaCO_3$ to pH 4.5.

*Marks the data with significant difference from the control reactor (*p-value* <0.05).

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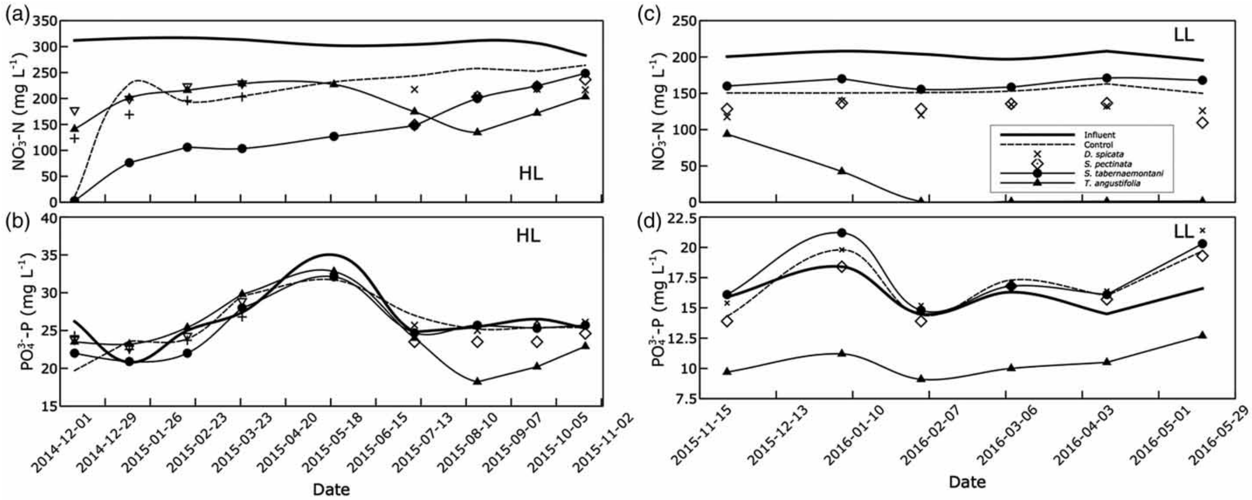

Figure 2 | Temporal trends of nutrient concentrations in wood-chip bioreactors. HL, high loading; LL, low loading. Influent and outflow $NO_3^- - N$ in HL (a), $PO_4^{3-} - P$ in HL (b), $NO_3^- - N$ in LL (c) and $PO_4^{3-} - P$ in LL (d).

water quality parameters, except for $NO_2^- - N$ and pH, in comparison with the control unit (Table 4, Figure 2(c), 2(d) and Figure 3). This planted unit resulted in average $NO_3^- - N$, $PO_4^{3-} - P$ and $SO_4^{2-} - S$ reductions of 88.4%, 34.4% and 21.5% respectively. Very high $NO_3^- - N$ reductions promoted by this species (minimum outflow concentration <1 mg L^{-1}) indicated successful denitrification of up to 99% and 6.2 g $NO_3^- - N$ day^{-1} reductions which were coupled with sudden increases in BOD_5 values (Figure 4(a)–4(c)).

Table 4 | Average parameters of the inflow (influent) and outflow solutions in wood-chip bioreactors receiving the synthetic greenhouse LL effluent

Parameter (unit)	Influent		Control	*S. taber.*	*S. pectinata*	*D. spicata*	*T. angustifolia*
$NO_3^- - N$ (mg L^{-1})	202.2 [4.9]	Out[a]	153.0 [4.6]	163.8 [6.0]	129.2 [9.4]	128.3 [7.9]	23.4 [34.9]*
		Red.[b]	24.3%	19.0%	36.1%	36.5%	88.4%
$NO_2^- - N$ (mg L^{-1})	1.2 [0.4]	Out[a]	1.4 [0.9]	1.1 [0.2]	1.1 [0.4]	1.0 [0.1]	1.1 [0.4]
		Red.[b]	–	8.3%	8.3%	16.7%	8.3%
$NH_3 - N$ (mg L^{-1})	5.5 [0.3]	Out[a]	0.5 [0.8]	0.9 [0.5]	0.0 [0.0]	0.2 [0.1]	4.4 [5.3]*
		Red.[b]	90.9%	83.6%	100.0%	96.4%	20.0%
TKN – N (mg L^{-1})	6.6 [0.9]	Out[a]	1.8 [0.8]	2.0 [0.8]	1.4 [0.4]	2.2 [0.4]	8.3 [5.5]*
		Red.[b]	72.7%	69.7%	78.8%	66.7%	–
$PO_4^{3-} - P$ (mg L^{-1})	16.0 [1.3]	Out[a]	16.9 [2.2]	17.5 [2.4]	16.3 [2.1]	17.5 [2.3]	10.5 [1.2]*
		Red.[b]	–	–	–	–	34.4%
$SO_4^{2-} - S$ (mg L^{-1})	129.6 [4.0]	Out[a]	132.3 [4.5]	132.7 [6.7]	131.6 [3.4]	131.5 [2.4]	101.7 [25.5]*
		Red.[b]	–	–	–	–	21.5%
Alk.[c] (mg L^{-1})	3.9 [1.4]	Out[a]	155.5 [10.9]	134.8 [14.4]	231.3 [38.8]	243.3 [25.2]	717.1 [177.5]*
BOD_5 (mg L^{-1})	3.1 [1.4]		5.2 [3.3]	3.8 [1.4]	2.6 [0.9]	4.1 [1.7]	50.4 [31.0]*
COD (mg L^{-1})	15.3 [3.0]		30.5 [3.8]	36.6 [3.2]	24.5 [3.4]	51.6 [3.3]	181.6 [77.0]*
pH	5.1 [0.6]		7.4 [0.0]	7.1 [0.1]*	7.5 [0.0]	7.3 [0.1]	7.4 [0.1]

SD values are given in brackets.
[a]The measured constituent in the outflow solution.
[b]Reduction with respect to the influent.
[c]Alkalinity as $CaCO_3$ to pH 4.5.
*Marks the data with significant difference from the control reactor (*p-value* <0.05).

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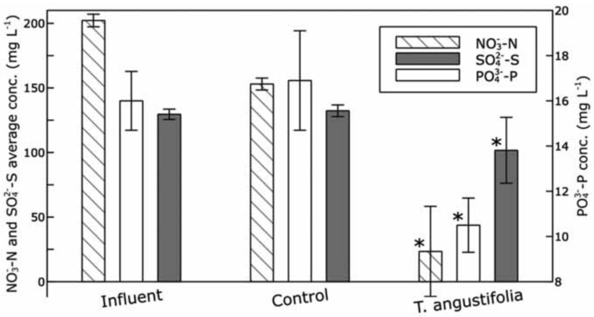

Figure 3 | Average performance of the wood-chip bioreactor planted with *T. angustifolia* in the LL period. Error bars: ±SD and asterisk (*): significant difference in comparison with the unplanted reactor (*p-value* <0.05).

As the nitrate ions were almost completely reduced, starting in February 2016 (Figure 2(c)), sulfate concentrations were also reduced (average 27.9 mg S L^{-1}) indicating that the anaerobic environment and absence of nitrate ions had provided favourable conditions for sulfate reducing bacteria. This was evident by the sulphide odour in the proximity of the bioreactor, together with the change of the outflow water colour, which turned murky with apparent white to grey particles accumulated in the drainage basin (potential precipitated sulphide). The near-complete nitrate removal followed by reductions in sulfate concentrations in the wood-chip bioreactors might be favourable for the greenhouse industry as the small levels of nutrients and salts enhance the potential of the greenhouse effluent for discharge and reuse respectively. The outflow solution's colour change and the precipitates may however be considered as drawbacks to potential water reuse.

The development of sulfate reducing conditions after complete denitrification in a streambed bioreactor has been previously reported (Shih *et al.* 2011), where the authors showed when sulfate reducing conditions were active, concentrations of methylmercury, a bio-accumulative toxicant, increased. The authors further suggested that maintaining a minimum residual $NO_3^-{-}N$ concentration of 0.5 mg L^{-1} would suppress the production of methylmercury. In our study, however, sulfate reduction in the *T. angustifolia* bioreactor was still evident when the outflow $NO_3^-{-}N$ concentrations were larger than 1.0 mg L^{-1}. In greenhouse settings, it can be argued that greenhouse effluent is less susceptible to mercury bound in the soil minerals and solids in agricultural farmlands and fields, which would be carried in the agricultural tile runoff before reaching the bioreactors. The risk of the methylation of the mercury entering the system via other pathways (e.g. wood chips from contaminated trees and water drawn from lakes that contain mercury from atmospheric deposition) should however be considered in the bioreactors.

The alkalinity level of the outflow solution was significantly higher in the *T. angustifolia* unit with an approximately 183-fold increase from the inflow. The

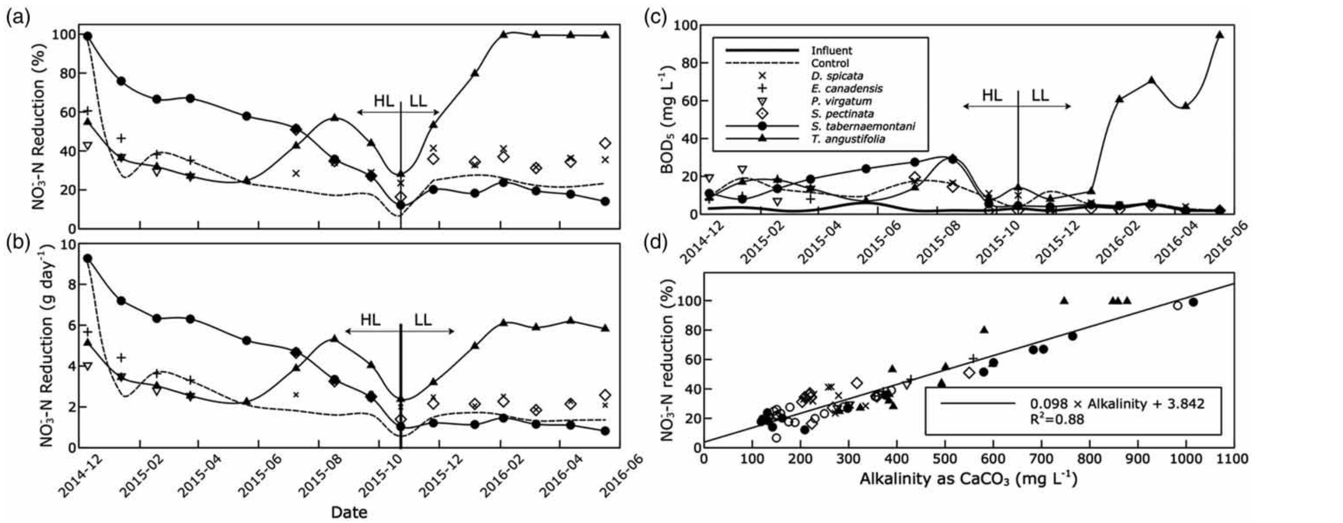

Figure 4 | Performance parameters of wood-chip bioreactors during the total operation time. HL, high loading; LL, low loading. Percentage $NO_3^-{-}N$ reduction (a), $NO_3^-{-}N$ mass reduction rate (b), influent and outflow BOD_5 values (c), and percentage $NO_3^-{-}N$ reduction vs outflow alkalinity (d: data points for control reactor in d are shown by empty circles; trendline: linear regression).

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connection between the alkalinity and denitrification in the wood-chip bioreactors, due to the production of bicarbonate in heterotrophic denitrification, and the application of the measured alkalinity as an indicator for bioreactor performance with particular respect to N_2O formation was recently studied (Jones & Kult 2016). The direct proportionality of the outflow solution's alkalinity to nitrate reductions for all the bioreactors throughout the entire operation of the wood-chip reactors is shown in Figure 4(d).

The percentage removal of nitrate in the *T. angustifolia* bioreactor (Figure 4(a)) had a rapid enhancement shortly after the beginning of the LL phase. This sudden increase is expected since the smaller nitrate concentrations in the inflow during the LL phase should result in higher percentage removal values assuming no significant change in the reduction capacity of the system. In addition, the mass-based reductions (Figure 4(b)) show higher nitrate mass removal rates after the LL period started (average HL: 3.6, LL: 5.4, max total duration: 6.2, min: 2.3 g NO_3^-–N day^{-1}). The increased denitrification rate observed in this unit was coupled with a rapid spike in the outflow BOD_5 of the reactor (Figure 4(c)).

The results of the wood-chip bioreactors show that during the HL phase, all the units were overloaded by excessive levels of nitrate concentrations, and the bioreactors were only able to reduce nitrate between 30% to 55%, likely as a result of limited biologically available organics from the wood chips and the plants' below-ground biomass. The decrease in inflow nitrate concentrations (LL phase) then coincided with very high levels of organic carbon in the form of BOD_5 that were made available, likely as a result of the vigorous growth of *T. angustifolia* and the microbial contribution of the plant's rhizosphere in contact with the wood chips, together with the fast decay of wood chips and the dead plant's biomass inside the bioreactor. The sudden increase in the natural supply of organic matter in this bioreactor was most likely the active driver in the almost complete denitrification, where the incoming nitrate concentration was the limiting factor in the reaction.

The active biomass growth of *T. angustifolia* may explain the phosphate removal via plant uptake by this species that stood out from the rest of the units (Figure 2(b) and 2(d) and Figure 3). However, the delayed onset of the phosphate reduction and simultaneous removal of phosphate and nitrate may indicate that phosphorus accumulating organisms or denitrifying phosphorus accumulating organisms (DNPAOs) were naturally developed in this reactor. Under anoxic conditions, the DNPAOs have been shown to use nitrate as electron acceptors for biological denitrification and phosphate removal (Yin *et al.* 2015). Further research is however needed to confirm the presence of these organisms in our bioreactors.

Over the total operation period of the wood-chip experiment (HL and LL phases), the bioreactor containing *T. angustifolia* resulted in an average nitrate removal of 22.5 ± 7.3 g N m^{-3} media day^{-1}, while the average removal amounted to 28.2 ± 5.5 g N m^{-3} media day^{-1} during the LL phase (Table 5). This observed reduction rate is over 25% greater than the highest previously reported rate of 22 g N m^{-3} media day^{-1} (Robertson *et al.* 2000; Schipper *et al.* 2010b). *S. pectinata* and *D. spicata* resulted in reductions of 11.5 ± 1.1 and 11.7 ± 1.2 g N m^{-3} media day^{-1} in the LL phase and 15.5 ± 6.2 and 13.7 ± 2.2 g N m^{-3} media day^{-1} in the HL phase respectively. As the nitrate removal performance of all the bioreactors except for *T. angustifolia* decreased in time, this species demonstrating the beneficial capacity of enhancing the denitrification efficiency in time.

Table 5 | Average NO_3^-–N removal rates, calculated from the data points during each experiment's operation time in wood-chip bioreactors

	$dm_{NO_3^- -N}$ (g m^{-3} media day^{-1}) Mass removal rate		$r_{NO_3^- -N}$ (mg L^{-1} day^{-1}) Zero-order removal rate		$k_{NO_3^- -N}$ (day^{-1}) 1st-order removal rate	
	HL[a]	LL[b]	HL[a]	LL[b]	HL[a]	LL[b]
T. angustifolia	18.7 [5.6]	28.2 [5.5]	32.1 [9.6]	48.3 [9.4]	0.14 [0.05]	1.04 [0.52]
Control	15.4 [12.3]	7.8 [0.8]	26.4 [21.0]	13.3 [1.3]	0.17 [0.27]	0.08 [0.01]

SD values are given in brackets.
[a]High loading.
[b]Low loading.

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CONCLUSIONS

The wood-chip denitrifying bioreactor planted with *T. angustifolia* showed consistent nitrate removal with an average reduction of 22.5 g N m^{-3} media day^{-1} and up to 99% treatment. The contributions provided by this species in the wood-chip bioreactor resulted in very high denitrification rates while nitrate concentration was the limiting factor in the LL phase. As with similar denitrification bioreactors with near-complete nitrate removal, sulfate reduction and production of sulfide compounds were evident which lead to the need for further assessment of the system for potential methylmercury formation.

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First received 1 December 2016; accepted in revised form 25 April 2017. Available online 9 May 2017

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