

Natural treatment of domestic wastewater and desert soil restoration using green soil amendments

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Abstract

This study evaluated the combination effect of soil amendments with sandy soil on wastewater contaminants removal, and the ultimate impact on soil restoration. A soil column experiment was conducted with sandy soil (CK) or soil amended with 4% (w/w) compost, biochar or maize straw. Wastewater infiltration was recorded, and alfalfa was planted subsequently. Results showed that amendment of biochar and straw significantly decreased domestic wastewater infiltration rate in sandy soil, with a reduction of 62 and 50% compared to CK. All combination of soil and amendments had removal ability for wastewater contaminants, as indicated by the sorption capacity and the resist for desorption. The largest sorption capacity was found in biochar-amended soil. Plant and soil microbes also played important role in contaminants removal. The amendment of biochar and compost significantly improved plant growth, microbial abundance and diversity. The plant biomass of compost and biochar treatments was 3.8 and 4.6 folds of CK. After plant harvest, straw and compost treated soil had increased soil aggregates compared to CK. It is concluded that sandy soil-organic amendments-plant system could be used as an efficient wastewater treatment strategy for nutrient and pathogen removal. Moreover, wastewater input and plant cultivation could improve structure of the coarse-sandy soil.

Introduction

There is an increasing world consumption of fresh water resources. The water scarcity in desert area is especially serious and the use of wastewater for irrigation is also common in water-scarce areas (Pedrero et al. 2010; Meena et al. 2016; Shilpi et al. 2018). It was suggested that 96% of the rural domestic wastewater and 50% of domestic wastewater in small city in China is untreated and reused for irrigation or directly discharged (Wu et al. 2013; Han et al. 2016), posing a great risk of pollution to soil, surface and ground water bodies.

Nutrients, salt, pharmaceuticals and personal care products, industrial additives and high concentrations of organic substances are the common contaminants in untreated domestic wastewaters (Angus et al. 2002; Zhang et al. 2015). Thus, the potential negative environmental impacts by using or discharging domestic wastewater include soil salinization (Ganjegunte et al. 2018), reduced soil infiltration rate (Tunc and Sahin 2015), biological contamination of pathogens (Akponikpe et al. 2011), changes in soil microbial functional

diversity (Arif et al. 2016) and input of N, P and endocrine (Huang et al. 2019) and heavy metal contamination (Frenk et al., 2014). However, due to the relatively poor technique and local economy of small and scattered populations in desert areas, an effective, low cost and environmental-friendly decentralized wastewater treatment strategy is urgently needed.

Nature-based wastewater purification systems have been reported as a feasible solution for scattered communities, small municipalities and non-connected dwellings where limited access is available to sewage networks (Martinez-Hernandez and Coauthors 2018). The application of nature-based system for these areas is also inspired in Europe (Martinez-Hernandez et al. 2020). And the system is usually used to treat secondary and tertiary wastewater (Andres and Sims 2013) for plantation, such as lawn and forest for biomass production. In this system, wastewater treatment is a combined effect of soil, plants, and subsequent thrive of microorganisms. The treatment efficiency of this system has been reported (Aronsson and Perttu 2011, de Miguel et al. 2014; Martinez-Hernandez et al. 2018). In such systems, the main processes of contaminant attenuation include physical filtration, sorption onto the soil, biodegradation and plant uptake. The nutrient uptake and sorption by vegetation and soil prevent contaminants from reaching groundwater. This ecosystem can apparently be served as nutrient and contaminants filter and sink, with the advantage of low costs, soil restoration, and vegetation recovery and reuse of water resources. However, desert soil has problems of large infiltration, low organic matter content, coarse texture, and poor water and fertilizer holding capacity (Van Asperen et al. 2014), thus is not a good sink for wastewater contaminants. As N and slat move easily in desert soil, and the possibility of leaching towards deeper levels is large when wastewater is applied (Liu et al. 2021), so as the other contaminants in wastewater.

The amendment with organic material may compromise the above deficits and enhance efficiency for wastewater treatment. When used for the purpose of wastewater treatment, the organic material is also named vegetation filter. Organic amendment to soil as a natural sorption media is proved in many studies to be an ecological and sustainable strategy. It could improve soil fertility, enhance crop production and microbial diversity in soil (Lazcano et al. 2013; Xu et al. 2019), contributing to organic matter pools and aggregate formation, with a range of agronomical, ecological and social benefits (Woolf et al., 2010). The organic amendments frequently used are crop residue, swine manure, compost and biochar. Crop residue such as straw from corn is a bio-product partly being returned to nature to increase soil organic matter content, thereby contributing to the formation and stabilization of soil aggregates and improvement of soil fertility. While compost is the stabilized outcome of the aerobic microbial biodegradation of biomasses (Hargreaves et al., 2008). Biochar is the by-product of pyrolysis process of organic biomass residues (Lehmann et al. 2003). It is mainly reported to improve soil physical properties, but may harm plant growth when using certain types of biochar (Liu. et al. 2017). Its porous structure and large surface area give the characteristics of large sorption capacity for various hazardous compounds such as nutrients (Pokharel and Chang 2019) and trace metals (Rechberger et al. 2019). However, hitherto the efficacy of different amendments in removal of wastewater contaminants is rarely studied. Also, the effectiveness of contaminants removal in a natural system with combination of desert sandy soil, organic amendments, and plants and microbes remains unknown.

In the current experiment, desert sandy soil was incorporated with straw, compost and biochar to evaluate the effect of different amendments on removal efficiency of wastewater contaminants; to test an effective, low cost and ecological wastewater treatment system; and to investigated the effect of the system on sandy soil restoration and vegetation recovery. To this end, the effects of amendment addition on desert soil water infiltration process; the transportation of wastewater contaminants throughout soil column including salt, *E. coli*, N, P and organic carbon were measured; the sorption and removal capacity of wastewater contaminants by selected amendments were compared, the plant rehabilitation and microbial community in desert soil were also analyzed. The result of this research could be served as an eco-approach for domestic wastewater treatment in scattered communities, small municipalities and non-connected dwellings.

2. Materials and methods

The experiment was conducted in a climate-controlled chamber. Columns were used to simulate the wastewater transport in desert soil. Cylinder column was 4 cm in inner-diameter and 110 cm deep (volume of $1.38 \times 10^3 \text{ cm}^3$) with 5 sampling holes arranged every 20 cm alongside of the column. Each column was filled to a dry bulk density of 1.43 g/cm^3 corresponding to a total of 1.8 kg dry soil. While the bottom of column was ended with 1 cm diameter drainage hole.

Four soil amendment treatments were included: straw (crop residue), biochar, compost and no amendment CK. The amendment was applied to the top soil layer of 5-45 cm with an application rate of 4% (w/w). The amendment was mixed thoroughly with soil before being packed into the column. The experiments were divided into three stages: infiltration with raw domestic wastewater at first, subsequently elution with fresh water and planting of alfalfa. The experiment was designed as complete randomized using four amending treatments, each with three replicates.

2.1 Materials

The soil used was sandy soil collected from Kubuqi Desert, Inner Mongolia, China. The soil was collected from a depth of 0-1 m in desert, and was air-dried. The soil was classified as sand, with 94.5% sand, 0.5% silt and 5% clay, water content 0.8%, organic matter content 0.02 g/kg, total N 0.012 g/kg, mineral N (NH_4^+ 0, NO_2^- 0 and NO_3^- 0.04) mg/kg, Olsen P 1 mg/kg, water soluble phosphorus 0.27 mg/kg, pH 8.19, bulk density 1.47 g/cm^3 , specific gravity 2.64 g/cm^3 , seepage velocity 15.37 mm/min, and porosity 44.25%. The pot holding capacity of column soil without amendment (CK) was 15.38%, and that for soil amended with compost, biochar and straw was 23.54%, 35.14% and 34.36%, respectively.

The wastewater used was domestic wastewater (WW) collected from wastewater treatment plants located in Peking University. The WW was not treated but was through primary sedimentation tank. The heavy metal contained in the above RW was lower than national standard for drinking water. The detected heavy metal in current WW was negligible (Cu, 0.006 ug/L; Zn, 0.002 ug/L; As, 0.004 ug/L; Cd, 0.000 ug/L; Pb, 0.000 ug/L). The composition analysis of the wastewater was shown in Supplemental table 1.

The amendments used in current experiment were straw, biochar and compost. The straw was maize residue having an organic matter content of 894.2 g/kg, total carbon 578.4 g/kg, total nitrogen 14.1 g/kg, drying at 45 to constant weight, grinded and sieved through 0.9 mm mesh. The biochar used was standard biochar produced from miscanthus straw pellets pyrolyzed at 700 degC in UK Biochar Research Center (UKBRC), University of Edinburgh, UK. Its total carbon of 791.8 g/kg and total N of 10.3 g/kg. The compost used was 3-month fermented mixture of maize straw and activated sludge at 20-25, then dried at 60 to constant weight, grinded and sieved through 0.9 mm mesh. The compost had an organic matter content of 712.2 g/kg, total carbon 395.1 g/kg, and total N 24.5 g/kg.

2.2 Experimental design

2.2.1 Wastewater infiltration and fresh water elution

To simulate wastewater process in desert soil and to reveal the sorption pattern of wastewater component by soil and amendments, column experiments of two stages were performed: wastewater infiltration for 4 days, followed by tap water leaching for 5 days. The ponding height of the wastewater was maintained at 4 cm above the soil surface in column by the elution bottle (Mariotte reservoir provided by JingRui ZeXiang instrument and equipment development Co., Ltd) to keep a constant pressure. The infiltration depth and quantity were recorded every 10, 30, 60 second until effluent run out from the bottom of the column. Then the bottom effluent was collected at 10, 20, 40 min, 1 h, 2 h, 1 day to 4 days. Afterwards, the effluent was changed to tap water and the water table was sustained the same level as above. The drainage was collected at 10, 20, 40 min, 1 h, 2 h, 1 day to 5days. The stable infiltration rates were measured at the time when it

reached a stable level after 60 min' infiltration. An organic shell in-between domestic wastewater and sand was observed and the shell was stirred up by glass rod at the time effluent collection of 2 h and the following every sampling of effluent.

2.2.2 Planting of alfalfa

Alfalfa (*Medicago sativa* L.) seeds were pre-germinated. One seedling was planted in each column. The plants were drip irrigated with wastewater to a 90% pot holding capacity by drip irrigations every second day. The drip equipment and plant growth condition were referred to Liu et al. (2021). The chlorophyll index of the leaves was measured with Chlorophyll Meter SPAD-502plus (Konica Minolta Optics, Inc.) one day before harvest. Plants were harvested 58 days after planting. Soil samples were sterilely collected at a depth of 20 cm. Plant samples (1g fresh weight) that used for surface *E. coli* analysis was collected 20 cm above soil surface with leaves and shoot together. Total biomass was the sum of shoot biomass and root biomass; root properties were measured with root weight density and root length density (methods referred to Liu et al. 2021)

2.3 Measurements and statistical analyses

2.3.1 Chemical analysis of effluent

The water samples were filtered through water membrane (0.45 μm). The data was obtained by averaging of 3 samples. The NO_3^- , NO_2^- and NH_4^+ were measured with spectrophotometer at 220 and 275 nm, 540 nm and 420 nm, respectively (Chinese Environment standards, HJ/T 346-2007). Total soluble salt (salt) content was measured by oven dry of the water samples. The detection of total phosphorus (TP) referred to Sissingh, (1971).

The sorbed wastewater component by soil and amendment was calculated, assuming that the component of amendment would not be released into the effluent:

Sorbed content (Cs) = sum of inlet content (Ci) – sum of effluent content (Ce) (1)

The percentage of wastewater component sorbed by soil and amendment = $\text{Cs}/\text{Ci} \times 100$ (2)

2.3.2 Plant chemical contents analysis

Plant samples were dried (60 °C until constant weight) and finely grounded. Plant P concentration was measured with spectrophotometer after ashing and digestion, addition of vanadate molybdate. Plant N concentration was measured using a combustion analyzer (2400 II CHNS; Perkin-Elmer). Plant N (P) uptake (mg/plant) was calculated as total N (P) concentration of plants (mg/g) multiplied by plant biomass (g/plant).

N remove by plant was calculated by the percentage of plant N uptake in N adsorption by soil (Cs):

Percentage of plant N remove = $\text{plant N uptake} / \text{Cs} \times 100$ (3)

The percentage of plant P remove was calculated the same way as for N.

2.3.3 Microbial analysis of effluent, plant and soil

The effluent, plant and soil samples were sterilely collected for microbial analysis immediately after sampling (within 12 h). Plant samples (1g fresh weight) were soaked in sterile vial with 0.005 M pyrophosphate buffer (pH=7) for bacterial extraction, afterwards, the diluted samples were shaken for 1 h to take supernatant for *E. coli* detecting using Tryptone Bile X-glucuronide Agar (TBX) (Van Damme et al. 2017). The surface *E. coli* of plant samples were calculated as the sum number of *E. coli* on the surface of aboveground plant (cfu/plant) by assuming that the *E. coli* was evenly distributed on plant surface. The *E. coli* of effluent was also measured with TBX Agar.

The genomic DNA for soil microbial community was analyzed by Illumina MiSeq platform (Illumina, San Diego, USA) according to protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Detailed procedures referred to Liu et al. (2021).

Soil from the column was collected for aggregation analysis after plant harvest. The water stable aggregates ([?] 0.25 mm) was detected by a wet-sieving procedure (Miller and Kemper 1965).

2.3.4 Statistical analysis

Statistical analysis was conducted using a one-way ANOVA Tukey test of variances at the significance level of $P = 0.05$ by SAS (9.3). The microbial community was analyzed using the platform of Majorbio I-Sanger Cloud.

3. Result and discussion

3.1 Wastewater infiltration

The infiltration rates of soil amended with different organic amendments are presented in Fig.1. Infiltration is a vital process of the hydrological course, and its quantification is a fundamental step in effective soil and water management (Parchami-Araghi et al. 2013; Lassabatere et al. 2010). High infiltration rate may benefit crop production in areas that receive regular and plentiful rainfall, yet, questionable for sandy soil in arid area. By comparing the stable infiltration rates and the time when drainage came out from soil columns, the results indicated that the amendment of biochar and straw significantly decreased domestic wastewater penetrability in desert soil. The time needed for water efflux from the bottom of soil column was extended from 32 min to 50 and 62 min in biochar and straw treatments, respectively, as compared with the CK. All the treatments reached a stable infiltration rate within 120 min. The stable infiltration rate with compost, biochar and straw treatments were reduced by 10, 50 and 62%, respectively, compared to the CK. Here, a reduced infiltration rate indicated improved water conservation in desert soil and furthermore an increased retention of wastewater in soil profile. The increased retention of wastewater prolonged the reaction time of contaminants within soil, following a possible increase of contaminants being adsorbed by soil or amendments, taken by plants or decomposed by soil enzyme and microbes.

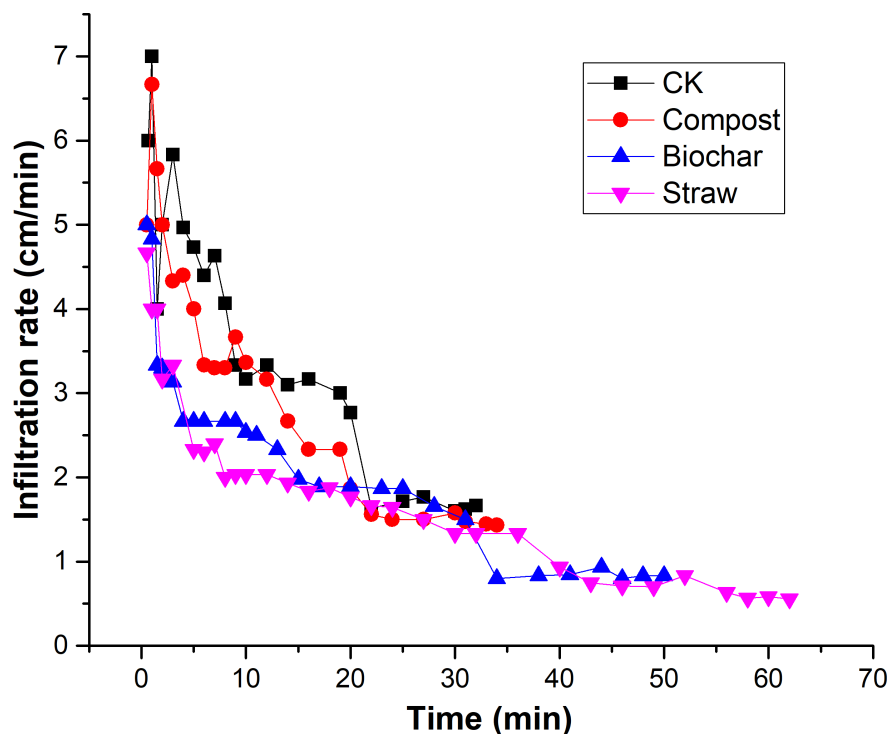


Fig.1 Domestic wastewater infiltration rates (cm/min) in sand soil subjected to different soil amendments (values are means).

3.2 Removal of wastewater component by soil and amendments

Vertical flow reactor used for removal of pollutants had been widely reported, e.g. Yu et al. (2020). The breakthrough curve and elution curve of different components are presented in Fig. 2. The breakthrough curves generally permit a good description the loading behavior of chemical component to be removed from solution in a fixed bed. Based on these two curves, the dynamic sorption and desorption process of wastewater contaminants by amendments could be illustrated.

The NO_2^- data was not presented here, as the NO_2^- content in all wastewater and effluent was negligible. The initially NH_4^+ was the main form of N in the tested domestic wastewater (see Supplemental table 1). Both NH_4^+ and NO_3^- in wastewater were ready to precipitate downward as the transportation of NH_4^+ and NO_3^- in soil solution is easy and sandy soil has little colloid to be absorbed to. However, a rapid decrease of NH_4^+ and NO_3^- was observed in the first 120 min (Fig. 2), which could be partly explained by the increasing thickness of organic matter shell in-between domestic wastewater and sand. The thickening organic shell would not only slow down wastewater infiltration rate, but also adsorb part of NH_4^+ . Besides, NH_4^+ can be easily converted to NO_3^- within soil by nitrification. Afterwards, the concentration of NH_4^+ in effluent had a step rise and eventually reached a relatively steady level similar to that of the influent wastewater, except for treatment of compost. This could attribute to the relatively high N content in the compost. The observed increase of NO_3^- in effluent could be converted from NH_4^+ by nitrification processes during infiltration, which was also evident in previous research (Martinez-Hernandez et al. 2020). Differing from NH_4^+ , NO_3^- from soil column effluent decreased rapidly within a day in all treatments. The removal of NO_3^- could be attributed to denitrification and dissimilatory NO_3^- reduction to NH_4^+ (as there was an increase in effluent NH_4^+ concentration). While anaerobic ammonium oxidation could be excluded, since this process can be inhabited by high concentration of organic matter (Nordstrom and Herbert 2018) from wastewater and high-carbon amendment input. When columns were subjected to elution, a great amount of NH_4^+ was washed out of soil column by tap water especially within the first 120 min. By day 3, the NH_4^+ concentration

in the effluent was almost zero in all treatments. Previous study has also addressed N removal via different filtration media in a rapid infiltration basin for wastewater treatment (Wen et al. 2020). The application of organic amendments with high content of carbon can provide electron donors for denitrification. For instance, woodchips have been used to treat high NO_3^- concentration wastewaters with promising results (Nordstrom and Herbert 2018; Schipper et al. 2010).

The total P concentration in the effluent increased with time in all treatments when subjected to wastewater infiltration (Fig. 2); then reached a constant but lower level in CK, straw and biochar effluent and a similar level in compost effluent compared to initial P level. Different from NH_4^+ , it took longer time for P to be washed out of soil column with a peek at 1 day except compost treatments, since P has higher reactivity than N, and binds easily to soil constituents (Frossard et al. 2000; Liu et al. 2015; Liu et al. 2018).

As amendments addition improves organic matter content in soil, a large amount of TOC in the drainage was detected with wastewater infiltration especially in the compost and straw treatments (Fig. 2). An accumulation of organic shell on the top layer of soil column was also observed with continuous wastewater inlet. The organic matter in the wastewater is also considered to be an important source for soils deficit in organic matter (Ganjegunte et al. 2018), especially the desert sandy soil in which both nutrition condition and soil structure is poor.

The salt concentration in wastewater used was low (see Supplemental table 1). Similar to N, soluble salt flued out easily from soil column along with wastewater irrigation. Most salt in soil column was washed out within a day (Fig. 2). Similar to other wastewater components, the *E. coli* in the wastewater could leach down to deep soil layers with wastewater irrigation and elution water. The transport of *E. coli* in the column in the straw treatment was a bit different from the others; even though there were no *E. coli* observed in the drainage in the first 10 min, but soon afterwards, a larger amount of *E. coli* was flued out compared with the other three treatments. Bacteria usually carry negative charge on their surfaces. Hydrophobicity, van der Waals forces and electrostatic repulsion are the main factors dominating interaction between bacteria and soil particles (Mills 2003).

Table 1 The percentage (%) of wastewater component sorbed by soil and amendments. Values are means (n=3). Significant differences are indicated by letters: Different small letters within a column indicate significant difference due to amendment method at $P < 0.05$.

	NH_4^+	TP	Salt	TOC	<i>E. coli</i>
CK	32.38 b	75.31 a	27.41 b	54.95 a	79.04 bc
Compost	21.77 c	35.81 b	19.23 c	21.80 c	84.66 b
Biochar	40.16 a	72.21 a	34.07 a	34.18 b	88.51 a
Straw	24.88 c	75.34 a	0.58 d	-113.34 d	73.04 c

Table 2 The percentage (%) of wastewater component washed out from column soil. Values are means (n=3). Significant differences are indicated by letters: Different small letters within a column indicate significant difference due to amendment method at $P < 0.05$.

	NH_4^+	TP	Salt	TOC	<i>E. coli</i>
CK	57.19 b	19.34 b	96.61 b	56.12 b	11.06 a
Compost	79.67 a	72.07 a	118.70 a	98.38 a	6.20 b
Biochar	38.81 c	20.51 b	89.45 bc	100.85 a	2.07 c
Straw	72.42 a	16.72 b	99.90 b	-32.68 c	7.06 b

The sorption and removal capacity of wastewater contaminants by selected amendments are compared in Table 1 and Table 2. The leakage of nutrients, especially N, can represent a limitation when natural based

wastewater treatment technology is properly operated (de Miguel et al. 2014). As the initial NO_3^- in the wastewater was also low, only NH_4^+ was used to evaluate the sorption and removal capacity. The sorption capacity for NH_4^+ , salt and *E. coli* (Table 1) was largest with biochar-amended soil as compared to other treatment. However, compared to CK, the incorporation of compost and straw to soil did not increase the retention of NH_4^+ , TOC and salt. This demonstrated that there was little sorption capacity of soil for the above wastewater component as the amount of N, salt and C in the amendments being washed out was not accounted. Still, the significant high sorption ability of biochar for N, salt and *E. coli* showed great potential for wastewater treatment.

By comparing the soil sorption rate for different component, the highest sorption rate was found with *E. coli* (>80%) and P (>70%, except compost treatments); while the sorption for N and salt was relatively lower. The high sorption rate of P was in accordance with previous findings that P has low transportation rate (Liu et al. 2015) and was easy to be adsorbed by soil and the high *E. coli* interception by soil indicated that soil might be a good treatment system for pathogen removal.

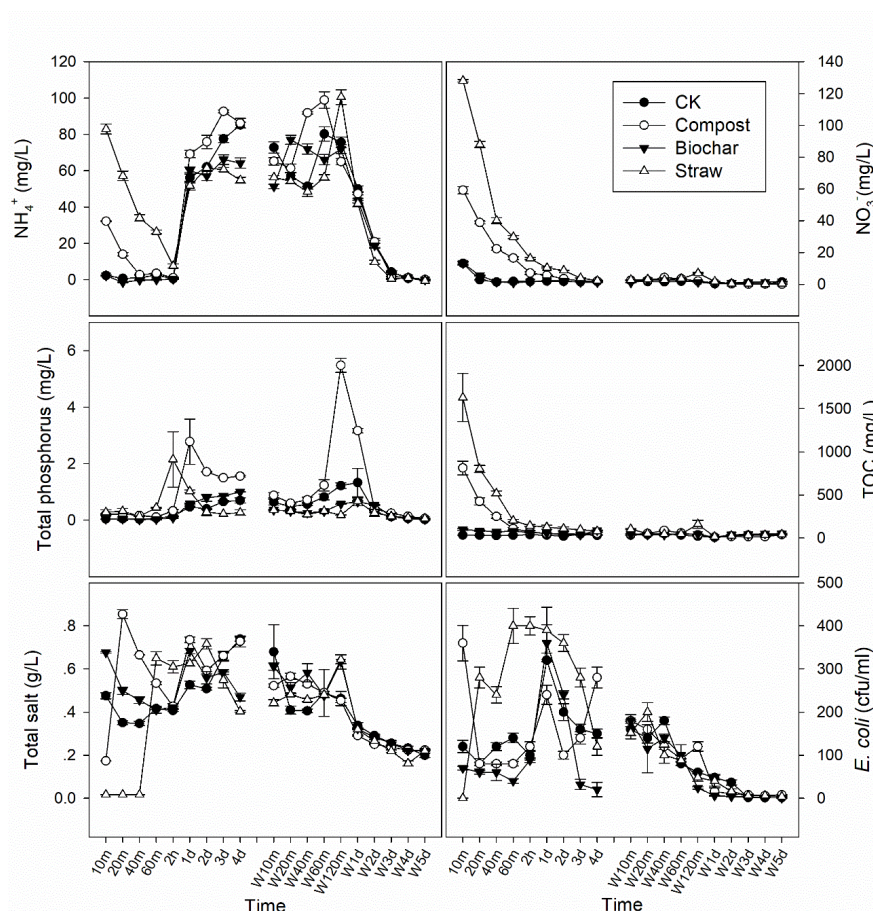


Fig. 2 NH_4^+ and NO_3^- (mg/L), total phosphorus (mg/L), total organic carbon (TOC) (mg/L), total salt (g/L) and *E. coli* (cfu/ml) concentration in effluent collected from the bottom of soil column that subjected to different soil amendments (values are means).

However, based on the elution curve (Fig. 2) and table 2, it could be seen that most of the wastewater component could be washed out to drainage, especially salt, NH_4^+ and TOC. While the washing out ratio of *E. coli* and P were much smaller, which further confirmed the high treatment efficiency of *E. coli* and P

in this natural wastewater treatment system. In this elution process, biochar also showed good performance in resisting of desorption, as indicated by the significantly lower percentage of NH_4^+ and *E. coli* than other soil amendments. Further removal of wastewater component in particular of N and TOC may attribute to plant and microorganism.

3.3 Plant biomass, root weight density and Chlorophyll content index

Alfalfa (*Medicago sativa* L.) is one of the most important cultivated forage species worldwide and it is widely planted in northwest China. Unlike annual crops, alfalfa is a deep-rooted perennial that able to remove NO_3^- from the subsoil beneath the rooting zone (Blumenthal and Russelle 1996) and its high adaptability, and protein content (Li et al. 2007) make it a high-quality feed. Removal of wastewater component by plant is due mainly to biomass incorporation. On the other way around, use of wastewater contributes resource recovery and has been successfully practiced in biomass production of olive trees, alfalfa, maize, napier grass and sunflower (Pedrero et al. 2020). The amendment of compost and biochar significantly improved plant growth (Table 3) compared with CK, with a biomass increment of 3.8 and 4.6 times, respectively.

Table 3 Plant total biomass (biomass g/plant), leaf area (LA cm^2 /plant) and Chlorophyll (SPAD), plant N uptake (N uptake mg/plant), plant P uptake (P uptake mg/plant), percentage of N remove by plant (%), percentage of P remove by plant (%), plant surface *E. coli* (surEcoli cfu/plant) and water stable soil aggregates[?] 0.25 mm (Aggregate g/column) that subjected to different soil amendments. Values are means (n=3). Significant differences are indicated by letters: Different small letters within a column indicate significant difference due to amendment method at $P < 0.05$.

	Biomass	SPAD	Nuptake	Puptake	Nremove	Premove	SurEcoli	Aggregate
CK	5.48 b	20.33 d	5.00 c	0.26 b	1.64	1.31	2 b	0.21 c
Compost	26.50 a	48.33 b	29.15 b	1.60 a	14.23	17.52	12 a	21.74 b
Biochar	30.68 a	62.00 a	39.52 a	2.08 a	10.52	11.47	0 b	0 c
Straw	8.33 b	30.67 c	9.95 c	0.54 b	5.57	3.33	5 b	65.57 a

The plant chlorophyll content index in the biochar treatment was the highest among all treatments, indicating a greater leaf greenness. The great plant biomass increment of compost and biochar treatments was clearly connected to the nutrition and water retention status of the soil column: 1) All the amended soil had a higher water holding capacity compared with CK, and the improved soil water holding capacity by biochar application has also been found by Sun et al. (2013); 2) Biochar addition to soil was found to be efficient in intercepting wastewater N and P (Table 2); 3) The significant high initial N content of compost can serve as nutrition for plant growth; 4) More water and nutrients were available for plant growth due a bigger root system with biochar and compost plants (Fig. 3).

The counts of *E. coli* on plant surface indicated that plant could hardly be contaminated by wastewater pathogen after two months' soil-plant treatment. It further confirmed that soil-plant system was efficient in pathogen removal. After plant harvest, the soil aggregation was analyzed. It was found that even though the original desert soil had no aggregation, the amendment of straw and compost increased water stable aggregates compared with biochar and CK treatments, which was a sign of soil structure formation. Usually the sparse vegetation on desert land was mainly due to lack of water. But even if irrigated, the soil with coarse-texture, high infiltration rate and low water and nutrient retention is difficult for plant survival. One way to increase water holding capacity of soil is to improve soil structure. The poor soil structural of desert sand was mainly due to its extremely low organic matter content, thus the application of compost and straw would contribute to the significant large soil aggregate formation. Besides, Bronick and Lal (2005) found that plant roots and their exudates could also enmesh and realign soil particles thus resulting in formation of soil aggregates.

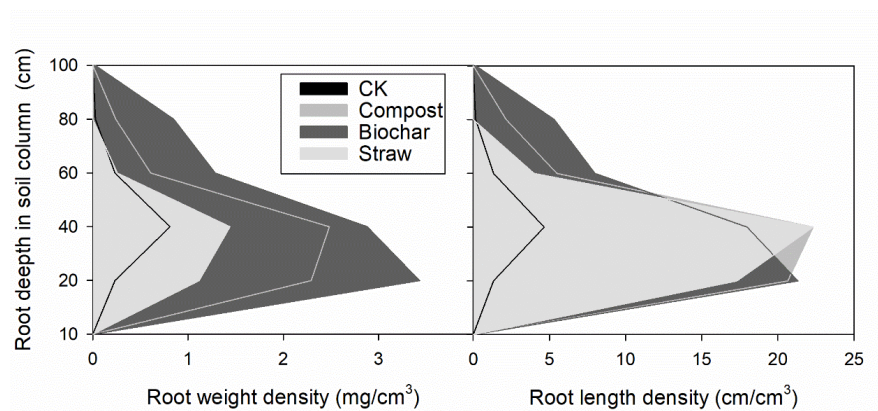


Fig. 3 Root weight density (mg/cm^3) and length density (cm/cm^3) alongside soil column that subjected to different soil amendments (values are means).

Compared with the CK, all soil amendments improved plant root growth (Fig. 3). Plant roots could reach to 100 cm depth in the biochar and compost amended soil column. The root was mainly distributed in the soil layer of 10-40 cm, which was also the layer of soil amendments. The biochar treatments had the largest root weight and length density, and the root biomass of biochar, compost and straw treatments was 7.8, 5.0, 2.3 times greater than that of CK. Thus, the cultivation of alfalfa in this wastewater treatment system could be a promising strategy to conserve water, stabilize shifting sand and control desertification in the desert area in northwest China.

Soil microbial community abundance and diversity after plant harvest

The composition of microbial communities plays important role in soil function. Clearly, the sand soil used in the current experiment is not a good microhabitat as there is rare aggregates, organic matter or roots. The change of microbial community due to soil amendment could provide important information for wastewater treatment. The microbial sequencing information and community diversity characteristics of soil irrigated with different wastewater were presented in Supplemental table 2. The indices of Sobs, Ace and Shannon indicated community richness and community diversity respectively. Microbial richness and diversity were largest in the biochar and compost amended soils, and smallest in the CK soil. Consistent with this, the enhanced pore structure and aeration by biochar addition, with improved physico-chemical conditions for retention and removal of nutrients and organic matter by plants and microbes had been reported by Ajayi and Horn (2017).

The microbial removal of wastewater component was mainly through decomposition. The original C/N in wastewater was 2.54. It is known that a C/N of 25:1 favoring microbial decomposition of organic matter in soil. Thus, the application of amendments with high C/N ratio in the current experiment would increase the N and P removal in wastewater treatment system, as once there is lack of biodegradable organic matter, the denitrification is inhibited and risk of NO_3^- leaching increase (Martinez-Hernandez et al. 2020). Moreover, the amendment of high-carbon organic matter can promote the activity of soil denitrifying bacteria by favoring anoxic conditions (Meffe et al. 2016). The predominant bacteria at phylum and genus levels by taxonomic analysis are shown in Fig. 4. In all wastewater irrigated soils, Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi were the dominant phyla, with Proteobacteria amounted for the largest proportion. The predominant genera in compost amended soil were *Rhodococcus*. And the most abundant genera in CK, biochar and straw amended soils were *Pseudomonas*, *Brevundimonas*, *Acidovorax*, respectively. The *Pseudomonas* are aerobic denitrifier and *Pseudoxanthomonas* possess excellent performance on nitrite removal (Meng et al. 2015). In addition, *Hydrogenophaga* accounted for 6% in the biochar soil (data was not presented), while these genera were not detected in other treatments. The growth of *Hydrogenophaga* could explain the high removal capability for N in biochar amended soil, as they are the major contributors to the

hydrogenotrophic denitrification process in wastewater treatment (Xing et al. 2018).

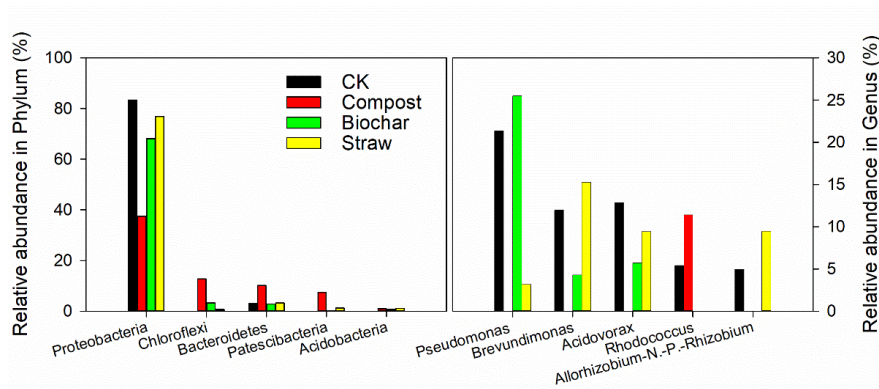


Fig. 4 The relative abundance of 16S rRNA gene reads at phylum level and genus level in soil subjected to different soil amendments.

Community heatmap analysis on Genus level of microorganisms under different soil amendments is illustrated in Supplemental figure 1 in order to reveal the similarities and differences of microbial community composition. It showed that soil amendment altered the bacterial composition. The biochar and CK soils had more similarity in microbial community structure, while the compost treatments were clearly distinct from the CK, biochar and straw treatments. The change of microbial community in the amended soil compared to CK could be a result of shifted physical and chemical composition due to amendments as well as the presence of alfalfa plant.

A liner regression analysis was done between the relative abundances of bacterial genus and soil physico-chemical (data of soil chemical analysis and correlation was not presented); and the relative abundances of bacterial genus and plant growth properties. It was found that soil organic matter, soil pH and plant biomass were the main predictors controlling bacterial community structure.

4. Conclusion

A natural wastewater treatment system using media of desert soil mixed with green amendments was tested in the current experiment, aimed to develop a low-cost wastewater treatment system, and a furthermore improvement of desert soil quality. Results showed that the system consisting sandy soil-green sorption product-plants could be a promising natural and low-cost strategy in wastewater treatment. The soil and amendments sorption, plant uptake and microbial decomposition in this system could help in understanding the mechanism of contaminant removal from domestic wastewater.

The amendment of biochar and straw could significantly decrease domestic wastewater infiltration rate in desert soil. The stable infiltration rate with straw, biochar and compost treatments were reduced by 62, 50 and 10% compared to CK.

The soil and amendment removal of wastewater contaminants was remarkable as demonstrated by increased sorption capacity and resistance for desorption for wastewater contaminants. The large sorption capacity for NH_4^+ , salt and *E. coli* in biochar amended soil indicated that biochar is a good soil amendment for wastewater treatment. Despite of soil and amendment combination, high sorption rate was found with *E. coli* (>80%) and P (>70%, except for compost treatments), while the sorption for N and salt was relatively lower. Thus, wastewater component can be washed out to drainage, especially with salt, NH_4^+ and TOC.

The efficiency of wastewater contaminants removal by plant and microbes were also evaluated. The amendment of compost and biochar significantly improved plant growth, microbial richness and diversity compared with CK. The biochar treatments also had the largest root weight and length density. The biomass increment of compost and biochar was 3.8 and 4.6 times of CK. High N removal capability by bacteria was also evident, especially with biochar-amended soil. After wastewater infiltration and plant harvest, soil collected from column showed sign of soil structure formation with increased water stable aggregates in straw and compost amended soil.

Thus, the system of sandy soil, organic amendment and plant has great potential as an efficient wastewater co-treatment strategy for nutrient and pathogen removal, and is applicable for domestic wastewater treatment in dry area with scattered communities, small municipalities and non-connected dwellings. Further system optimization according to local climate and field experiment should be carried out to confirm the findings.

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