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Effects of Sewage Sludge Application on Plant Growth and Soil Characteristics at a *Pinus sylvestris* var. *mongolica* Plantation in Horqin Sandy Land

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Abstract: The application of domestic sewage sludge (SS) may affect plant growth and soil quality through altering nutrient availability. However, the effect of SS application on the plant-soil system in sandy soils is poorly understood. In this study, we established SS application treatment plots (SL, 25 t ha⁻¹) and control treatment plots without sewage sludge application (CK, 0 t ha⁻¹). SS was applied to the soil surface of a Mongolian pine (Pinus sylvestris var. mongolica) plantation in Horqin Sandy Land, Inner Mongolia, China, to assess its potential effects on plants and soil. We analyzed tree growth performances (tree height, basal diameter, and diameter at breast height), understory traits (species diversity, coverage, and aboveground biomass), soil physical and chemical parameters (nutrient content, dissolved organic carbon, soil water content, bulk density, pH), and proxies of ecosystem services (soil organic carbon and total nitrogen stocks). The results showed that SS addition not only significantly increased soil nutrient contents, but also markedly enhanced aboveground productivity and plant coverage. Specifically, SS addition decreased soil bulk density and increased concentrations of soil organic carbon, total nitrogen, and total phosphorus and mineral nitrogen, and it also increased soil carbon and nitrogen stocks. Furthermore, the addition of SS significantly increased soil dissolved organic carbon contents and enhanced the fluorescence intensities of dissolved organic carbon components (humic acid-like and UV fulvic acid-like) in the topsoil (0-5 cm). This study provides evidence that SS is an acceptable, and possibly preferred organic fertilizer for improving the soil quality and tree-grass growth of Mongolian pine plantations.

Keywords: plantation; understory; soil improvement; sewage sludge application; soil carbon/nitrogen stock; dissolved organic carbon; Horqin Sandy Land

1. Introduction

Sewage sludge (SS) is an inevitable byproduct of the process of wastewater treatment [1,2]. Based on a recent study, approximately 39 million tons per year of SS are produced due to the dramatic growth in wastewater treatment plants in China [3]; this is 2–3 times the amount of sludge production in the United States [4,5]. The vast amount of sludge produced is drawing the attention of researchers worldwide [6–8]. How to dispose of SS has become a common issue. At present, SS has been processed by the use of several technical methods, such as land utilization, biochar, building materials, landfills, and incineration [7,9–14]. The most suitable disposal practice for SS is land utilization, where it is used to improve soil fertility and fulfill circular economy requirements. Because



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). of the high organic matter, nitrogen (N), phosphorus (P), nutrient contents, and abundant microelements (e.g., Cu, Zn, Fe, etc.), SS is recognized as a useful source of fertilizer or soil amendment and is widely applied in terrestrial ecosystems [2,15,16]. According to the reports of Wei et al. [3], land utilization is the main method of SS disposal in China, accounting for close to 30%. Additionally, the main disposal method for SS in the United States is also land utilization, and is up to approximately 47% [17]. Furthermore, due to the hazardous substances (heavy metals, organic pollutants) in SS, most countries have established national regulations that control the application of SS land disposal, such as the United States' Code 40 of the Federal Regulations, Part 503, and the European Union's Council Directive 86/278 [1,18]. In China, the application of SS onto farmland and forest-land is also regulated by GB 4284-2018 and CJ/T 362-2011, respectively. Recently, a more restrictive limitation (NYT525-2021) has been put in place that prohibits using SS as a raw material for organic fertilizer, which has caused serious challenges for SS disposal in terms of land utilization. Hence, a more acceptable, cost-effective, and environmentally feasible approach for SS land utilization needs to be considered.

Degraded lands (such as tailing land, sandy land, etc.), which contain poor nutrients, offer an opportunity for applying SS [19–21]. Many studies have reported that the application of SS could promote soil fertility, tree growth, and vegetation productivity in European and American forests [22–25], as well as in Asian forests [26]. Indeed, the application of SS positively increases the contents of soil organic carbon, total nitrogen, available nitrogen, and total phosphorus required by plants [27], resulting in an increase in the wood production of plantations [28] and changes in the understory vegetation community and biomass [19]. More importantly, SS application on forest plantations (e.g., windbreak and sandbreak forest, tailing reclamation forest, and timber forest) can prevent some hazardous substances from entering the food chain and reduces the potential pollution risk for humans and livestock. Although tree growth and soil quality responses to SS application have been widely studied on various types of forestlands in tropical, subtropical, and temperate regions [24,26,28,29], few studies have investigated the impact of SS on plantations in arid and semiarid regions, especially on semiarid sandy land, which limits our evaluation of the effectiveness of SS application on sandy plantations.

Mongolian pine (*Pinus sylvestris* var. *mongolica*), due to its low temperature resistance and drought tolerance, has become one of the main afforestation tree species in the Three-North region, and was successfully introduced from Hulunbuir Sandy Land to the southeast of Horqin Sandy Land in 1955 [30,31]. Mongolian pine plantations have been widely established in Horqin Sandy Land in Northern China to slow down wind speed and fix sandy soil [32]. However, recent studies have reported that a dieback and mortality phenomenon of Mongolian pine has emerged, probably due to competition for limited nutrients and water within these pure Mongolian pine plantations [33,34]. Long-term competition will impact the stability and sustainability of Mongolian pine plantations within these regions.

Nitrogen and water are considered to be important limiting factors for plant growth performances in the southeast of Horqin Sandy Land [35,36]. A good possibility for enhancing nutrients and soil water availability in the region could utilize the SS originating from wastewater treatment plants. At present, in pot experiments, some studies have found that short-term (140 days) application of SS promoted the growth of two-year-old Mongolian pine seedlings [37]. However, studies regarding the effects of SS on the tree–grass–soil system in Mongolian pine plantations are still lacking, especially regarding the mid- and long-term effects of tree growth, understory species diversity and biomass, soil labile carbon components, and inorganic nitrogen content following successive applications of SS to the immature forests of Mongolian pine on sandy land.

Thus, more work is needed to understand how SS application influences the plant performance and sandy soil physical and chemical properties of Mongolian pine plantations. In this study, we have hypothesized that plant growth and soil quality are both promoted under SS application. Therefore, we have conducted the following experiment: (i) to study the growth performances of Mongolian pine and herbaceous community characteristics under the application of SS; (ii) to examine the responses of soil properties and soil organic carbon and nitrogen stocks to the application of SS onto Mongolian pine plantation soil in a semiarid region.

2. Materials and Methods

2.1. Site Description

The study was conducted in Daqinggou Ecological Research Station, located in the southeastern region of Inner Mongolia, China (42°54' N, 122°24' E; about 260 m above sea level) (Figure 1). The climate is classified as a temperate climate. The average annual temperature in this area is about 6 °C, with a 1780 mm average of annual potential evaporation, and 154 frost-free days. The average annual precipitation is approximately 450 mm. The main vegetation types at the station are Mongolian pine plantations, Mongolian pine sparse woodland, poplar (*P. beijingensis*) shelterbelts, and grasslands. The vegetation in the study site consists of a Mongolian pine plantation, which was established in 2002 by planting four-year-old seedlings within a 4.0 m \times 3.0 m planting grid. At the beginning of the SS application experiment in April 2015, the average tree height was 4.9 m, and the average diameter at breast height (DBH) was 13.7 cm. Dominant understory herbs in this location were dominated by Artemisia scoparia, Cannabis sativa, Setaria arenaria, Leymus *chinensis, Lespedeza daurica,* and *Lespedeza hedysaroides*. The major soil type is sandy soil (91% sand, 5.0% silt, and 4.1% clay), developed from aeolian parent material and characterized by loose structure [38]. The soil is deficient in organic carbon, nitrogen, and phosphorus, whose concentrations at the surface soil are $2.0-3.0 \text{ g kg}^{-1}$, $0.2-0.3 \text{ g kg}^{-1}$, and $0.05-0.15 \text{ g kg}^{-1}$, respectively [39,40].



Figure 1. Study area (Daqinggou Ecological Research Station, Ganqika Town, Tongliao City, Inner Mongolia, China; southeastern edge of Horqin Sandy Land).

2.2. Sewage Sludge

The SS was obtained from the Ganqika domestic wastewater treatment plant (Inner Mongolia, China). The wastewater treatment plant has an anaerobic–anoxic–oxic activated sludge process (A2/O process), with a capacity of 5×10^4 m³ d⁻¹, and the wastewater is mainly derived from domestic sewage (accounting for approximately 80% of the total). Before the experiment began, the collected SS was aerobically composted, air-dried, ground, and sieved by a 2 mm mesh. According to the Chinese Standards (CT/T221-2005), the total content of metals (Zn, Cu, Cr, Cd, Ni, Pb, As, and K) was assessed by methods of digestion

with HCl (37%), HNO₃ (68%), and H₂O₂ (30%). The contents of Zn, Cu, Cr, Cd, Ni, Pb, and K were determined using an atomic absorb spectrometer (PerkinElmer PinAAcle 900T; PerkinElmer Inc., Waltham, MA, USA), and the content of As was analyzed by an atomic fluorescence spectrometer (Haiguang Instrument AFS-9700A; Haiguang Instrument Co., Ltd., Beijing, China). The concentration of PAHs was measured using HPLC (Agilent Model 1200; Agilent Technologies Inc., Santa Clara, CA, USA), after extraction of the SS samples with hexane and dichloromethane (C_6H_{14} and CH_2Cl_2 , analytical grade) [9]. The water content of the fresh sludge sample was measured using the gravimetric method. The main characteristics of the SS are presented in Table 1.

Parameter	Unit	Mean Concentration in the SS	Limits
pН		7.4	
Dry matter	%	13.3	
Organic matter	%	77.7	
Total nitrogen	%	2.6	
Total phosphorus	%	1.0	
Total potassium	%	0.6	
NH4 ⁺ -N	$ m mgkg^{-1}$	1198.6	
NH_3^N	$mg kg^{-1}$	12.9	
Zn	$mg kg^{-1}$	341	<3000
Cu	$mg kg^{-1}$	65.7	<1500
Cr	$mg kg^{-1}$	69.9	<1000
Cd	$mg kg^{-1}$	0.5	<20
Ni	$mg kg^{-1}$	16.6	<200
Pb	$mg kg^{-1}$	11.9	<1000
As	$mg kg^{-1}$	63.4	<75
Benzo (a) pyrene	$mg kg^{-1}$	0.08	<3
PAHs	$mg kg^{-1}$	1.2	<6

Table 1. Main chemical characteristics of sewage sludge (SS) and standard limits established in China regarding the quality of sludge used in forestland (CJ/T 362-2011).

2.3. Experimental Design

Six random plots of 11.0 m \times 3.0 m were established at the Mongolian pine plantation (consisting of approximately 1.2 ha). There were two treatments, with three replications per treatment: sewage sludge application treatment with a rate of 25 t ha⁻¹ (SL) on a dry weight basis according to the standard (CJ/T 362-2011) and the control treatment, without sewage sludge application (CK). The prepared SS was spread at the end of April, from 2015 to 2017, on the soil surface after removing the understory plant litter from each plot.

2.4. Vegetation Survey

The growth performances of *Pinus sylvestris* var. *mongolica* were evaluated in terms of tree height (H), basal diameter (BD), and DBH in April and October 2020. The height of each tree was measured manually with the help of a holometer, while BD and DBH were determined by calipers at the same time. All measurements were carried out for eighteen trees in all plots. The growth increments of every tree were calculated by data collected from two measurements taken during 2020, according to Equations (1)–(3):

$$H_{\rm i} = H_{\rm oct} - H_{\rm apr} \tag{1}$$

$$BD_{i} = BD_{oct} - BD_{apr}$$
⁽²⁾

$$DBH_{i} = DBH_{oct} - DBH_{apr}$$
(3)

where H_i , BD_i , and DBH_i are tree height increment (m), basal diameter increment (cm), and diameter at breast height increment (cm), respectively. H_{oct} and H_{apr} , BD_{oct} and BD_{apr} , and

 DBH_{oct} and DBH_{apr} are tree height (m), basal diameter (cm), and diameter at breast height (cm), respectively, for October and April.

Data concerning the herb layers in the PM plantation were collected during the growing season. Two random quadrats, which included both the open-canopy position and the under-canopy position in each plot, were selected to investigate the herb species diversity and aboveground biomass in late August 2020 (the peak period of plant growth). Within each 0.5 m × 0.5 m quadrat, all herbaceous information, including the vegetation species, amounts, and coverage, were identified. The aboveground biomass was then harvested by clipping to ground level with a pair of scissors. Clipped grass from each quadrat was collected in paper bags, dried at 60 °C for 72 h, and weighed. Species diversity and indicators of herbage were expressed by Simpson's diversity index (D) (Equation (4)) and Species richness index (R) (Equation (5)), respectively, for each treatment, SL and CK, using the following equations [35,41]:

$$D = 1 - \sum P_i^2 \tag{4}$$

$$R = i \tag{5}$$

where P_i is a ratio between the number of species, *i*, and the total number.

2.5. Soil Collection and Laboratory Analysis

Soil samples were collected at depths of 0–5 and 5–10 cm after the herb investigation. Ten soil cores were randomly sampled with a soil auger (2.5 cm diameter) from the two 0.5 m \times 0.5 m quadrats in each plot. The soil samples of each depth layer were mixed into one composite sample. Twelve composite soil samples (2 treatments \times 2 depths \times 3 replicates) were transported to the laboratory within 24 h in a plastic foam box with ice bags. Subsequently, after removing litter, root, and debris with tweezers, the soil samples were sieved through a 2 mm mesh and divided into two sub-samples. One sub-sample was air-dried in the laboratory and used to determine pH, and the remnant was ground to pass through a 0.25 mm sieve and was used for physical and chemical analysis, i.e., soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) contents. The other sub-sample was stored at 4 °C for analyzing soil water content (SWC), NO₃⁻–N, NH₄⁺–N, and dissolved organic carbon (DOC) contents. In addition, three soil cores were collected randomly from each layer for the determination of soil bulk density (SBD) using stainless soil cores, 5 cm in diameter and 100 cm³ in volume.

Both physical and chemical analysis of the soil samples were carried out in triplicate for all the parameters. Soil pH was measured in a 1:2.5 air-dried soil/water suspension using a pH meter (Hach senslON3; Hach Co., Loveland, CO, USA). SOC was analyzed by the method of K₂Cr₂O₇ concentrated H₂SO₄ oxidation. TN and TP were determined by Auto Analyzer III (Bran+Luebbe AA3, Bran+Luebbe GmbH, Hamburg, Germany) after high-temperature digestion using concentrated H₂SO₄ and copper sulfate (CuSO₄) [42]. Soil NO₃⁻–N and NH₄⁺–N concentrations were determined by the methods described by Fan et al. [40]. Briefly, a 20 g fresh soil sample was extracted with a 50 mL 2 mol L⁻¹ KCl solution, shaken at 25 °C for 30 min, and then analyzed by AutoAnalyzer III. SWC was determined by oven-drying the fresh soil at 105 °C for 24 h. SBD was calculated from the ratio between soil dry weight and soil volume.

The soil organic carbon stock (SOCS, kg m⁻²) and soil total nitrogen stock (STNS, kg m⁻²) were calculated for each layer according to Equations (6) and (7):

$$SOCS_i = C_i \times B_i \times D_i / 100$$
 (6)

$$STNS_i = N_i \times B_i \times D_{i/100} \tag{7}$$

where C_i , N_i , B_i , and D_i represent the organic carbon content (g kg⁻¹), total nitrogen content (g kg⁻¹), bulk density (g cm⁻³), and soil layer thickness (cm) of the *i*th layer, respectively; i = 1 and 2.

Soil DOC was extracted by adding 50.0 mL of deionized-H₂O to 5.00 g of soil in a 100 mL centrifuge tube [43]. The suspension was shaken on an orbital shaker for 60 min at room temperature, centrifuged at 6000 r min⁻¹ for 30 min, and filtered with a filter of 0.45 μ m pore size. These filtrates were analyzed for organic carbon contents using a total Organic Carbon Analyzer (Jena Multi N/C3100; Analytik Jena AG, Jena, Germany). Fluorescence excitation–emission matrix spectra (EEMS) measurements of DOC were determined by a Cary Eclipse spectrofluorometer (Varian EL0507-3920; Varian Co., Palo Alto, CA, USA) with the following conditions: the excitation (Ex) ranged from 220 to 400 nm and the emission (Em) ranged from 300 to 600 nm in 5 nm increments. Instrumental parameters were as follows: excitation and emissions slits, 2.5 nm; averaging time, 0.1 s; and scan speed, 720 nm min⁻¹.

2.6. Statistic Analysis

The means and standard deviations of three replicates were calculated. Student's *t*-test was used for determining the differences of tree growth characteristics and the herbaceous species diversity, coverage, and aboveground biomass between the SL and CK treatments. A two-way analysis of variance (ANOVA) procedure was used to test the effects of sewage sludge application on plant growth and soil physical and chemical parameters, and to determine the interactions between treatment and soil layer using the general linear models (GLM) procedure. When the interaction was significant, a one-way ANOVA procedure was used to determine the differences between sewage sludge application and CK, and the two soil layers. Least significant difference (LSD) analysis was used to determine the relationships between all variables. The significance level, α , was set at 0.05. All statistical tests were performed with SPSS for windows, Version 16.0., Chicago, SPSS Inc.

3. Results

3.1. Effects of SS Application on Tree Growth

An increasing trend was observed in the height, DB, and DBH of all trees in the six plots during the period April to October, 2020. The height increments of Mongolian pine were 0.60 ± 0.04 and 0.48 ± 0.06 m during the growing season for treatments SL and CK, respectively. The increments in the DB (SL, 1.39 ± 0.09 ; CK, 0.94 ± 0.22 cm) and the DBH (SL, 0.68 ± 0.14 ; CK, 0.39 ± 0.06 cm) of trees were also estimated. The results showed that SS application significantly promoted the growth of Mongolian pine (Figure 2). The height increment, DB increment, and DBH increment of Mongolian pine in SL were, respectively, 1.26, 1.48, and 1.77 times greater than those in CK. The differences in these growth performances of Mongolian pine between treatments SL and CK were significant (p < 0.05).



Figure 2. Effects of sewage sludge (SS) application on Mongolian pine growth. (a) Height increment (m), (b) Basal diameter increment (cm), and (c) DBH increment (cm) of the tree. SL, SS application treatment; CK, control treatment without SS application. All the values are mean (\pm SD) for each variable, measured in all three replications (*n* = 3) within each treatment. * *p* < 0.05.

3.2. Effects of SS Application on Species Diversity, Coverage, and Aboveground Biomass of the Understory

There were no significant differences in Simpson's diversity index, species richness, or coverage between the SL and CK treatments (p > 0.05). Simpson's diversity index and species richness in the SL plots were lower than those in the CK plots (Table 2). In contrast, understory coverage in the SS application treatment ($48.33 \pm 22.68\%$) was greater than that in the CK treatment ($36.33 \pm 10.97\%$). In the SL treatment, Simpson's diversity index and species richness decreased by 42.31% and 30.77%, respectively, and coverage was increased by 24.83%. SS application also increased aboveground biomass by 44.07%. The difference in aboveground biomass between treatments SL and CK was significant (p < 0.05).

Table 2. Effects of sewage sludge (SS) application on Simpson's diversity index, species richness, coverage, and aboveground biomass within a 0.5 m \times 0.5 m quadrat.

Treatment	Simpson's Diversity Index	Species Richness	Coverage (%)	Aboveground Biomass (g m ⁻²)
SL	0.29 ± 0.21	3.00 ± 0.87	48.33 ± 22.68	176.27 ± 31.11
CK	0.51 ± 0.15	4.33 ± 1.89	36.33 ± 10.97	98.59 ± 32.51
Student's <i>t</i> -test	ns	ns	ns	*

SL, SS application treatment; CK, control treatment without SS application. Values are means \pm SD for each variable determined in three replicated plots within each treatment. * *p* < 0.05; *ns*, not significant.

3.3. Effects of SS Application on Soil Physical and Chemical Properties

At both the 0–5 and 5–10 cm layers, SWC and pH increased through SS application (p < 0.01 and 0.05, respectively), and SBD had a significant decrease from 1.52 to 1.43 g cm⁻³ and from 1.52 to 1.43 g cm⁻³, respectively (p < 0.05; Table 3). The mean values of SOC, TN, and TP in the topsoil (0–5 cm) were significantly higher than those in the subsoil (5–10 cm) in the same treatment, except for TN content. The variations in SOC at the 0–5 and 5–10 cm layers ranged from 3.68 to 5.61 g kg⁻¹ and from 2.59 to 3.11 g kg⁻¹, respectively (Table 3). SS application significantly increased the contents of SOC and TN, but not TP. In addition, the SL treatment induced changes in soil C:N ratio and N:P ratio. SS application decreased C:N ratio and significantly increased N:P ratio in the soil layers. There were no significant treatment × soil layer interactions on soil physical and chemical properties, such as SBD, pH, SWC, SOC, TN, TP, C/N, and N/P (Table 3).

Table 3. Effects of sewage sludge (SS) application on soil bulk density (SBD), soil water content (SWC), pH, SOC, TN, TP, C/N, and N/P at the 0–5 and 5–10 cm soil layers in the Mongolian pine plantation.

Treatment	Soil Layer (cm)	SBD (g cm ⁻³)	SWC (%)	рН	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)	C/N	N/P		
SL	0–5 5–10	$\begin{array}{c} 1.43 \pm 0.03 \\ 1.49 \pm 0.05 \end{array}$	$\begin{array}{c} 6.14 \pm 0.99 \\ 5.01 \pm 0.58 \end{array}$	$\begin{array}{c} 6.87\pm0.11\\ 6.99\pm0.09\end{array}$	$\begin{array}{c} 5.61 \pm 1.35 \\ 3.11 \pm 0.51 \end{array}$	$\begin{array}{c} 0.51 \pm 0.12 \\ 0.36 \pm 0.08 \end{array}$	$\begin{array}{ll} 0.51 \pm 0.12 & 0.15 \pm 0.03 \\ 0.36 \pm 0.08 & 0.09 \pm 0.01 \end{array}$		$\begin{array}{c} 3.4\pm0.3\\ 4.0\pm0.4\end{array}$		
СК	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		$\begin{array}{c} 6.55 \pm 0.13 \\ 6.71 \pm 0.09 \end{array}$	$\begin{array}{c} 3.68 \pm 0.15 \\ 2.59 \pm 0.02 \end{array}$	$\begin{array}{c} 0.31 \pm 0.06 \\ 0.29 \pm 0.05 \end{array}$	$\begin{array}{c} 0.11 \pm 0.02 \\ 0.08 \pm 0.02 \end{array}$	$\begin{array}{c} 12.2\pm2.7\\ 9.2\pm1.4\end{array}$	$\begin{array}{c} 2.8\pm0.4\\ 3.5\pm0.2\end{array}$			
Two-way ANOVA analysis											
Treatment (T)		*	*	**	*	*	ns	ns	*		
Soil layer (L)		ns	ns	*	**	ns	ns *		*		
T × L		ns	ns	ns	ns	ns ns		ns	ns		

Values are means (n = 3) and SD for soil physical and chemical parameters. * p < 0.05; ** p < 0.01; ns, not significant. SL, SS application treatment; CK, control treatment without SS application.

3.4. Effects of SS Application on Soil NO₃⁻–N, NH₄⁺–N, and DOC

The application of SS showed more increases in the soil NO_3^--N , NH_4^+-N , and DOC contents of SL (14.96–22.74, 4.09–8.87, and 81.23–86.99 g kg⁻¹, respectively) compared with CK (0.30–0.61, 0.87–1.31, and 70.17–72.43 g kg⁻¹, respectively) (Figure 3a–c). Soil

NO₃⁻–N and NH₄⁺–N contents were significantly higher in the topsoil (0–5 cm) than in the subsoil (5–10 cm) (p < 0.01 and 0.01, respectively). However, there was no difference in DOC content between the two soil layers in all the treatments. The interactions between sewage sludge application and soil layer were significant for soil NO₃⁻–N and NH₄⁺–N (p < 0.01 and 0.05, respectively) (Figure 3a,b), but not for soil DOC (Figure 3c).



Figure 3. Effects of sewage sludge (SS) application on (**a**) soil NO₃⁻–N content, (**b**) NH₄⁺–N content, and (**c**) soil DOC content at 0–5 cm and 5–10 cm layers in the Mongolian pine plantation. SL, SS application treatment; CK, control treatment without SS application. The values are the mean \pm SD (*n* = 3). *** *p* < 0.001; ** *p* < 0.01; * *p* < 0.05; *ns*, not significant.

To understand the change of DOC characterization, we measured the chemical composition of DOC by three-dimensional fluorescence spectra. These data provide valuable information on how the SS application affected the nature of DOC. The fluorescence excitation–emission matrix spectra (EEMS) contour maps of the soil DOC of the different treatments and soil layers are shown in Figure 4. It displays two main peaks: peak A at Ex/Em of 300–330/410–430 nm (humic acid-like fluorescence peak) and peak B at Ex/Em of 230–250/400–440 nm (UV fulvic acid-like fluorescence peak) (Figure 4a). As shown in Figure 4a–d, the soil DOC fluorescence intensities of two peaks decreased obviously with increasing depth. Upon the addition of SS, there is significant enhancement of the fluorescence intensities of soil DOC.



Figure 4. Three-dimensional fluorescence excitation–emission matrix spectra (EEMS) contour maps of soil DOC. (**a**) SL, 0–5 cm; (**b**) SL, 5–10 cm; (**c**) CK, 0–5 cm; and (**d**) CK, 5–10 cm. SL, sewage sludge (SS) application treatment; CK, control treatment without SS application.

3.5. Effects of SS Application on SOC and TN Stocks

SS application resulted in a significant (p < 0.05) increase in SOC stocks and TN stocks at both the 0–5 and 5–10 cm soil layers, compared to CK (Figure 5a,b). In particular, the SL treatment significantly improved SOC stock and TN stock in the topsoil (0–5 cm) by 43% and 55%, respectively. The SOC stocks were markedly higher in the topsoil than in the subsoil (p < 0.01). In contrast, TN stocks did not differ significantly (p > 0.05) between the 0–5 and 5–10 cm soil layers (Figure 5b). There were no significant treatment × soil layer interactions on SOC stocks and TN stocks.



Figure 5. Comparison of (**a**) SOC stocks and (**b**) soil TN stocks between sewage sludge (SS) application (SL) and without SS application (CK) at depths of 0–5 cm and 5–10 cm in a Mongolian pine plantation. The values are the mean \pm SD (n = 3). ** p < 0.01; * p < 0.05; ns, not significant.

4. Discussion

Our objectives were to investigate the responses of plant growth (trees and understory vegetations) and sandy soil characteristics to SS application in a semiarid Mongolian pine plantation ecosystem. The results confirmed that: (1) SS promoted tree growth, species diversity, coverage, and the aboveground biomass of understory; (2) SS increased soil nutrients concentrations, SOC stock, and TN stock. We discuss the results below and provide some new insights into the reasons for these responses.

4.1. Responses of Plant Growth Performances to SS Application

SS, often called "biosolids", is a useful resource of organic matter, nitrogen, phosphorus nutrients, and certain microelements that have positive effects on plant growth and biomass production [19,44]. The tree growth was estimated by various growth parameters, such as total height and diameter at breast height, etc. [45]. For example, Rodriguez et al. [24] investigated the effects of composted pulp-mill sludge on 17-year-old loblolly pine (Pinus *taeda* L.) plantations and found that the sludge application of 80 t ha⁻¹ resulted in significant increases up to 24% and 37% of stem diameter and height of the plants, respectively. Additionally, Rigueiro-Rodríguez et al. [46] showed significant tree height increases of Pinus radiata D. Don trees by applying a certain dose of anaerobically-digested SS as a fertilizer. Abreu et al. [28] found that the wood volume of the *Eucalyptus* tree, calculated by the height and the diameter at breast height, yielded a statistically larger increments of 44 m³ ha⁻¹ year⁻¹, due to the recommended sludge application (8, 15, and 23 Mg ha⁻¹, respectively). Our results showed that aerobically-composted SS application significantly increased the mean annual height, basal diameter, and DBH increments of Mongolian pine (Figure 2a–c). The results are similar to those described by Xu et al. [47] for poplars (*Populus* \times *euramericana* 'Guariento'), applying annual soil fertilization and reporting the largest average increase in DBH of 11.1% with a compost sewage sludge application of 15 t ha⁻¹ year⁻¹. Other authors, such as Hu et al. [48], recorded 161% and 145% increases of Mongolian pine tree height and basal diameter due to soil amendments applied to the

soil at an eight-year-old plantation. These results demonstrate that the application of SS as a fertilizer to improve tree growth in plantations is possible in poor nutrient areas.

Furthermore, the most significant variation in understory vegetation in the forest with sludge addition was the enhancement of coverage [49]. Moreover, in the SS fertilization of forests, it has been demonstrated that the understory biomass is increased [50–52]. An obvious observation regarding understory herbs in the Mongolian pine plantation during this study period was that there were significant differences between the SL and CK treatments, especially during summer (Figure 6). Our results show that the understory herbs that had the addition of SS were greater in coverage and aboveground biomass than CK (Table 2). In particular, an SS application of 25-ton dry weight per ha significantly promoted the aboveground biomass. Tandy et al. [53] similarly proved that the biosolids had positive effects on vegetation cover and biomass at a post-industrial sandy site. Additionally, Basta et al. [54] found that the biosolid treatments promoted a greater production of plant biomass and much more vegetative cover than in the control plots. The stimulating effects of SS were attributed to its large amounts of nutrients, which are important for plant growth [19]. In contrast, increased soil fertility with SS may affect the herb community responses. As reported by Gagnon et al. [55], the sludge reduced diversity due to the increased growth of grasses, without and differences in richness. Mohamed et al. [56] found that SS, as a soil amendment in a *Larix decidua* plantation, decreased species richness in the third year of the experiment. This was also found in this study (Table 2) after 3 years addition of SS and 3 years cessation of SS inputs. These results might be explained by the nutrients of the sludge accumulating in such nutrient-poor soils during 3 years of annual application, especially increasing the N availability in soil. A previous study by Yu et al. [57] showed that N was a primary limiting factor in this area. Thus, adding N-rich SS is a benefit for nitrophiles, and therefore leads to a reduction in diversity [35,55,58,59]. This result is consistent with the "self-thinning hypothesis" [60].



Figure 6. Growth status of understory herbs in Mongolian pine plantation during growing season: (a) SL, 25 t ha^{-1} (dry weight) composted sewage sludge application plot; (b) CK, control treatment without SS application plot.

4.2. Responses of Soil Properties to SS Application

Our results showed that soil bulk density significantly decreased after three years of continuous application of sewage sludge (Table 3), confirming the important role of sludge. Lower bulk density was also observed more in the topsoil than in the subsoil. These results are due to the higher content of organic matter, which is beneficial for soil aggregation and soil porosity [61]. Studies have also reported that organic amendment addition decreased soil bulk density [62,63]. Table 3 shows that the soil water content and pH value after SS addition were significantly higher than those of CK, probably because of changes in soil porosity structure and organic matter mineralization rate induced by the abundant organic nutrients in SS [64,65]. In our study, we found that soil water content

was positively correlated with SOC (r = 0.881, p < 0.01) and negatively correlated with bulk density (r = -0.725, p < 0.01) (Table 4). Furthermore, the SS used in the study was alkalescent in pH (7.4) and had a high content of organic carbon (Table 1). Consequently, the addition of the sludge resulted in modifying faintly acid sandy soil to a neutral pH (Table 3). These results coincide with Eden et al. [66], who reported a positive effect of organic waste on soil water in sandy soil, and with Ferreiro-Domínguez et al. [51], who found positive effects of SS on soil pH.

Table 4. Pearson correlation coefficients for relationships between plant performances and soil properties in the Mongolian pine plantation after sewage sludge application.

	н _i	BD _i	DBH _i	D	R	С	AB	pН	SBD	SWC	SOC	TN	TP	NO3 ⁻ - N	NH4 ⁺ -N	DOC
Hi	1	0.500 *	0.564 *	-0.675 **	-0.539 *	0.500	0.326	0.253	-0.077	0.003	0.130	-0.049	0.083	0.269	0.106	0.145
BD;		1	0.604 **	-0.308	-0.330	-0.458	-0.342	0.152	-0.106	-0.148	-0.107	-0.052	-0.102	0.056	-0.180	0.185
DBĤ _i			1	-0.399	-0.261	0.001	-0.122	0.213	-0.262	0.039	0.070	0.078	0.073	0.248	0.056	0.436
D.				1	0.850 **	-0.433	-0.331	-0.179	0.038	0.116	0.015	0.137	0.185	-0.311	-0.374	-0.202
R					1	-0.280	-0.350	-0.058	-0.170	0.063	-0.089	0.128	0.188	-0.272	-0.309	-0.199
С						1	0.449	0.207	-0.017	0.149	0.219	0.170	0.268	0.274	0.294	0.089
AB							1	0.649 *	-0.205	0.356	0.229	0.281	0.161	0.600 *	0.427	0.442
pH								1	-0.266	0.494	0.205	0.525	0.219	0.713 **	0.474	0.394
ŜBD									1	-0.725 **	-0.684 *	-0.795 **	-0.737 **	-0.709 **	-0.656 *	-0.815 **
SWC										1	0.881 **	0.950 **	0.897 **	0.738 **	0.640 *	0.586 *
SOC											1	0.875 **	0.910 **	0.708 **	0.647 *	0.645 *
TN												1	0.881 **	0.789 **	0.673 *	0.625 *
TP													1	0.594 *	0.533	0.549
NO ₂ -N														1	0.881 **	0.836 **
NH4 ⁺ -N															1	0.723 **
DOC																1

 H_i , height increment; BD_i , basel diameter increment; DBH_i , diameter at breast height increment; D, Simpson's diversity index; R, species richness index; C, coverage; AB, aboveground biomass; SBD, soil bulk density; SWC, soil water content. * p < 0.05; ** p < 0.01.

Soil nutrient element concentrations are important factors affecting soil health and plant growth [67]. Generally, sandy soil in semiarid areas is low in soil organic carbon, nitrogen, and phosphorus contents. SS can be used as a soil amendment, due to it containing abundant organic matter, essential plant nutrients, and various microbes [25,68]. In this study, the contents of SOC, TN, and TP in surface sandy soil increased after SS application (Table 3), which demonstrates that the SS had positive effects on the nutrient deficient soil of a Mongolian pine plantation in a semiarid region. Moreover, there were significantly positive correlations between SOC and TN, SOC and TP, and TN and TP (r = 0.875, p < 0.01; r = 0.910, p < 0.01; r = 0.881, p < 0.01, respectively) (Table 4). As expected, this finding could be related to the higher rates of organic matter, TN, and TP in SS (Table 1). Similar results have been reported by Chen et al. [69] and Carabassa et al. [70], who observed that SS addition showed increases in the contents of SOC, TN, and TP, and thus positively improved the soil fertility. Sardans et al. [71] pointed out that inputs of C, N, and P are the major processes that decide the stoichiometry of these nutrient elements in various ecosystems. Although SOC, TN, and TP contents were all altered by SS addition in our study, the decrease of C/N and increase of N/P indicated that microorganisms mineralized the organic carbon fractions and left a surplus N and enhanced P mineralization rate after SS was added to the soil [72].

Furthermore, the contents of inorganic nitrogen (NO₃⁻–N and NH₄⁺–N) at both the 0–5 and 5–10 cm layers were significantly increased as a result of SS application (Figure 4a,b), which is consistent with several other studies by Higashikawa et al. [73] and Song and Lee [65]. The reason for this was possibly the higher contents of NH₄⁺–N and NO₃⁻–N in the SS (Table 1). In addition, NH₄⁺–N was the major form of inorganic N in the SS. However, SS application over several years led to a sharp reduction in the soil concentration of NH₄⁺–N. On the contrary, the content of NO₃⁻–N was markedly increased. It was believed that soil NH₄⁺–N converted to NO₃⁻–N under the process of nitrification, when N was more available after SS input. Asik et al. [74] also, in an incubation experiment, demonstrated change patterns where soil NH₄⁺–N content decreased and NO₃⁻–N content increased with sludge applied from 0 to 160 t ha⁻¹ over a period of 150 days. In this study, we found that soil NO₃⁻–N and NH₄⁺–N were significantly positively correlated with soil water content (r = 0.738, p < 0.01; r = 0.640, p < 0.05, respectively) and significantly nega-

tively correlated with soil bulk density (r = -0.709, p < 0.01; r = 0.656, p < 0.05, respectively) (Table 4). Usually, nitrification is an aerobic process that is related to soil conditions such as soil moisture, aeration, etc. [41,75,76].

Consequently, these results indicate that SS application altered soil physical and chemical properties at both the 0–5 and 5–10 cm soil layers. In addition to higher soil moisture and soil pH, and higher SOC, TN, TP, and DOC contents and N/P, lower bulk density and C/N were observed in the SS application plots of the Mongolian pine plantation. Our results provided evidence that major soil properties were greatly sensitive to SS and highlighted the importance of improving the fertility of sandy soils when assessing SS application consequences at plantations.

4.3. Responses of SOC, TN Stocks, and DOC Content to SS Application

SOC and TN stocks are common concerns as regards soil improvement [77–79]. We found that SS, in the form of aerobic compost, was a source of the organic nutrients utilized in sandy soils of the Mongolian pine plantation and could significantly increase SOC and TN stocks in the 0–5 and 5–10 cm layers (Figure 5a,b). Enhancements of SOC and TN stocks in soils after fertilization with various organic compounds have been observed by many studies, especially in the surface soil layer [80,81]. Placek-Lapaj et al. [82] performed studies with SS application in crop soil producing pineapple and found an increase in the TOC stock and TN stock in the soil (0–60 cm) with the application of 8 and 2 t ha⁻¹, respectively. Similarly, a long-term field experiment conducted by Paetsch et al. [83] showed that urban waste composts (organic waste compost, green waste, and SS compost) enhanced SOC and N stocks after successive addition for a period of 15 years. Regarding SOC and N stocks, they are also influenced by many other driving factors, e.g., climate, land use/management, topography, soil properties, vegetation, etc. [84–88]. Thus, investigations on dynamic changes in SOC and N stocks are expected to intensify in the future.

Soil DOC, one of the labile fractions of SOC, plays a vital role in terrestrial ecosystem carbon cycling due to its high solubility, mobility, bioavailability, and reactivity [89–91]. In our study, the soil DOC increased in the amended soil, in agreement with the results from other investigations [92,93]. This uptrend in the content of DOC was mainly due to a large amount of dissolved organic matter (DOM) introduced into the soils with the addition of SS [94]. Generally, DOC is a class complex organic compound containing a small content of low molecular weight material (e.g., monosaccharide, oligosaccharide, fatty acid, aromatic acid, and amino acid, etc.) and a larger content of high molecular weight material (e.g., fulvic acid and humic acid), with complex structures [95]. A three-dimensional contour graph of DOC, obtained by fluorescence spectra, clearly presents the intensity of fluorescence with excitation and emission wavelengths, which is used for indicating the degree of DOC content. Our results revealed both similar and unique fluorophores on the fluorescence excitation–emission matrix spectra (EEMS) contour maps of top- and sub-soil DOC under different treatments (Figure 4a–d). The characteristic signatures of the DOC components showed contour maxima at Ex/Em of 300–330/410–430 nm (Peak A) and Ex/Em of 230–250/400–440 nm (Peak B). According to studies reported elsewhere, peak A and B are related to humic acid-like material and UV fulvic acid-like compounds, respectively [96,97]. Moreover, the results of the fluorescence intensities of the soil DOC coincided with those of DOC contents, which were confirmed by Liu et al. [98].

4.4. Implications for Relationships between Soil Properties and Plant Performance

In the present field experiment, SS was successively added to the sandy soils of a Mongolian pine plantation for three years. Consequently, the woodland utilization of SS resulted in an improvement of the soil properties, such as modifying soil pH, bulk density, and organic matter, and dramatically increasing NO₃⁻–N. Specifically, the soil pH and NO₃⁻–N were positively correlated with the aboveground biomasses in the understory (r = 0.649, p < 0.05; r = 0.600, p < 0.05, respectively). The amendment of sandy soil fertility also promoted tree height and herbaceous coverage, even though there were no significant

relationships with those indicators of soil nutrition (Table 4), which is consistent with previous reports [65,99]. Ferreiro-Domínguez et al. [51] also observed the effects of SS application (conversion into TN amount of 50 or 100 kg ha⁻¹) on soil chemical properties that resulted in positive impacts on the understory production and tree growth in a *P. radiata* D. Don plantation.

Unlike the results of Duan et al. [100], there were significantly negative relationships between herb diversity and woody growth parameters. We found that the height increment of Mongolian pine was negatively correlated with Simpson's diversity and Species richness (r = 0.675, p < 0.01; r = 0.539, p < 0.05, respectively) (Table 4). One reason for this is that the addition of N-rich SS into soil caused a reduction in herb diversity [35,55]; another reason is that there might be competition for nutrients and water at the upper soil layer between tree and herb species, because Mongolian pine is a shallow-rooted species with about 85% of its root distribution at a 40 cm soil depth [101–103]. Sunlight levels under canopies are often low in dense plantations, which may also be a factor in the negative tree–grass relationships [104]. In general, our results demonstrated that the application of SS not only benefited the growth of Mongolian pine trees within the plantation, but was also a beneficial sandy soil amendment regarding soil C and N stocks in a semiarid sandy ecosystem.

5. Conclusions

The application of aerobically-composted SS (25 t ha^{-1}) to a sandy soil that is deficient in nutrients promoted Mongolian pine growth (height, basal diameter, DBH) and understory performance (aboveground biomass and coverage) in a semiarid area. Three years of SS application clearly improved soil nutrient availability. Moreover, soil physical and chemical properties, i.e., SBD, SWC, pH, SOC, TN, and inorganic N, were significantly improved. Furthermore, both soil carbon stock and soil nitrogen stock were increased. SS application greatly increased the concentration of soil DOC and enhanced the fluorescence intensities of soil DOC components (humic acid-like and UV fulvic acid-like) in the topsoil. Overall, our results suggest that SS is possibly a beneficial organic fertilizer for improving soil quality and tree-grass growth in Mongolian pine plantations. In the future, we should focus on the changes in soil DOM dynamics (e.g., DON) and the structure and function of soil bacterial communities caused by SS application in Mongolian pine plantations. In order to further verify the safety of SS application, it will be necessary to undertake a long-term study for identifying plant-soil interactions under different doses of SS application, and to determine the cumulative effects of the heavy metals and organic pollutants in SS on plants, soil, and underground water.

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