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RESEARCH ARTICLE

Effects of filter mud applications on growth, physiological characteristics, and nutrient transfer pattern of sugar beet seedlings

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ABSTRACT

Filter mud is an industrial waste produced by sugar crops during the sugar production process, which contains N, P, K, organic matter, and a variety of trace elements required for plant growth. Therefore, a soil cultivation experiment was devised to verify the effects of applying diverse proportions of filter mud on the growth and physiological variations of sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) seedlings. For this study, sugar mill filter mud equivalent to 1%, 3%, 5%, 7%, 9%, 11%, and 13% of the dry weight of neutral black soil was used. Sugar beet seeds were planted in soil with filter mud and their growth, nutrient, and physiological indexes are measured after they grew into sugar beet seedlings. After the application of filter mud, the growth status of sugar beet seedlings increased significantly compared to the non-application of filter mud. Soil application of filter mud increases the content of inorganic N, available P, available K, and organic matter in the soil. And the most pronounced growth of sugar beet seedlings was achieved at 7% of the applied filter mud. In addition, the content of chlorophyll, transpiration rate, net photosynthetic rate, stomatal conductance, and phosphoenolpyruvate carboxylase activity all increased significantly. As a result, it was determined that filter mud from sugar beet waste was an effective soil improver material, a certain percentage of filter mud can promote the growth of sugar beet seedlings. This has important implications for the improvement of sugar beet yields and the recycling of agricultural wastes.

Key words: Beet filter mud, *Beta vulgaris* subsp. *vulgaris*, growth, nutrient, physiological indexes.

INTRODUCTION

Sugar beets (*Beta vulgaris* L. subsp. *vulgaris*) grow mainly in the colder regions of the Northern Hemisphere, which are produced in more than 40 countries. Sugar beets are also grown on a large scale in northern China (Geng and Yang, 2015). Sugar beet is an important sugar crop in China, and thus is widely grown for sugar production. The agricultural waste produced by sugar factories during the sugar production process from sugar crops is filter mud.

Since the composition of the filter mud is derived from the sugar crop before processing at the sugar mill, the filter mud contains N, P, K, and organic matter, as well as large amounts of other nutrients (Elsayed et al., 2008). These nutrients are needed for plant growth. Hence, the recycling of filter mud can significantly reduce the waste of these nutrients. In addition, beet waste that is not cleaned up promptly can cause pollution to the environment. Therefore, the direct discharge of filter mud as waste is both a waste of resources and a hazard to the environment. The comprehensive utilization of filter mud is an important part of the reuse of waste resources and the sustainable development of agriculture. Sustainable agriculture means using biological resources to maintain soil fertility (Berecz et al., 2005). Application of organic waste rich in nutrients to the soil, such as animal manure (Pandey and Chen, 2021), crop straw (Xu et al., 2021), green manure (Gross and Glaser, 2021), and the filter cake (Gonçalves et al., 2021) is the current agricultural practice of providing nutrients to plants. Organic waste should be recycled sustainably, and humans should also use natural resources in a way that identifies and reduces environmental risks (Rayment, 2005).

Filter mud has been used in some countries and regions such as Argentina, Australia, Brazil, India, Pakistan, Swaziland, and South Africa (van der Poel et al., 1998). And previous studies have focused on filter cake and its effect on sugar cane, but very little has been reported on filter mud and its effect on sugar beet. By-products of the sugarcane industry, such as filter mud and filter cake, are often used as a source of nutrients and soil amendments (Aljabri et al., 2021). The content of N, P, and K in soil could be increased by applying sugarcane filter cake alone or in combination with fertilizer (Kaur et al., 2005).

As filter mud showed greater potential in improving the growth properties of sugarcane (Abubakar et al., 2022), it was, therefore, necessary to design an experiment to test whether sugar beet filter mud could promote the growth of sugar beet. The current study aimed to explore the transformation of sugar beet filter mud from an unwanted by-product to a useful soil amendment. In this study, the soil cultivation experiment was devised to verify the effects of applying diverse proportions of filter mud on the growth and physiological variations of sugar beet seedlings by sowing sugar beet seeds. Despite the positive impact of chemical fertilizers on crop yields, they can also threaten environmental health. This dilemma makes it imperative to prioritize alternative agricultural practices that improve soil fertility by maintaining ecological balance or that help reduce chemical inputs over traditional agricultural practices (Asri, 2022).

MATERIALS AND METHODS

Plant material paragraph and growth conditions

Sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) is a biennial herb in the genus *Beta*, family Chenopodiaceae. The sugar beet cultivar selected for this experiment was 'KWS1176'.

Sugar beet seedlings were grown in a greenhouse at Heilongjiang University. During the growth of sugar beet seedlings, the conditions of greenhouse culture were: daily light for 14 h and 26 ± 1 °C, night for 10 h and 20 ± 1 °C, relative humidity 40% ~ 50%, and light intensity 700 $\mu mol \cdot m^{-2} s^{-1}$. Soil samples and filter mud samples were collected from the northeast of China. Black soil (Mollisols, USDA) came from Hulan Campus of Heilongjiang University, and filter mud came from Yi 'an Sugar Factory in Heilongjiang Province. The physicochemical properties of neutral black soil and filter mud are shown in Table 1.

In a design with seven replicates, eight treatments were implemented: A control group (without filter mud) and seven groups were mixed with different proportions of filter mud and neutral black soil (proportion of filter mud were 1%, 3%, 5%, 7%, 9%, 11%, and 13% respectively). In the experiment of sugar beet cultivation, 800 g evenly mixed soil were put into each pot, 20 coating seeds were evenly put and then covered with 100 g soil and water with a modified 1/2 Hoagland nutrient solution. Every treatment received an equal amount of water at daily regular times to obtain the same soil humidity, and every treatment has shifted the position regularly to ensure consistent lighting. After sugar beet seedlings grew to the first pair of leaves, four plants of the same growth were kept in each pot and harvested after 20 d of cultivation.

Table 1. Physicochemical properties of neutral black soil and filter mud at the experimental site, China.

Indicators	Neutral black soil	Filter mud
Inorganic N, mg kg^{-1}	39.9467	218.7943
Available P, mg kg^{-1}	35.3565	185.6585
Available K, mg kg^{-1}	209.3215	243.4473
Organic matter, %	2.5198	7.0212
pH	7.3867	9.2633

At harvest, a sample of three plants was randomly taken from each experimental pot. Vernier calipers were used to measure plant height, leaf length, and leaf width. The leaf area was calculated using WinRHIZO Reg2003b software (Regent Instruments, Quebec, Canada). An analytical balance was used to weigh the single above-ground fresh weight and single above-ground dry weight of the plants.

Physiological indexes

Chlorophyll content and net photosynthetic rate, and other indicators were determined in fresh sugar beet leaves. The chlorophyll content was measured using atomic absorption spectrophotometer as described by Zhao et al. (2013). Transpiration rate (T_r), net photosynthetic rate (P_n), and stomatal conductance (g_s) were measured using a hand-held photosynthesis system (CI-340, CID Bio-Science, Camas, Washington, USA). A representative sample of three plants was randomly taken from each experimental pot to measure phosphoenolpyruvate carboxylase (PEPC) activity, nitrate reductase (NR) activity, and glutamine synthetase (GS) activity in leaves. The PEPC activity was determined using atomic absorption spectrophotometer, GS activity was determined using atomic absorption spectrophotometer according to Miflin and Lea (1977), and NR activity in plant leaves was measured by the ex vivo method described by Song et al. (2017).

Nutrient

Total N content in sugar beet seedlings was determined by Kjeldahl using the method described by Wang et al. (2017), and total P was determined using the methods of ammonium molybdate by Wang et al. (2017). The flame spectrophotometer (FP640, Shanghai Precision & Scientific Instrument, Shanghai, China) was used to determine the content of total K (Pi et al., 2016). The content of inorganic N, available P, available K, organic matter, and soil pH was determined by Lu (2000).

Statistical analysis

All data were analyzed by ANOVA in a completely randomized design model using the SPSS 22.0 statistical software program (IBM, Armonk, New York, USA). Differences in treatments were assessed by Duncan's test at the 0.05 probability level. In this experiment, at least three independent replicates were analyzed. The image was processed using Prism 8 (GraphPad Software, San Diego, California, USA).

RESULTS AND DISCUSSION

Effect on growth indicators of sugar beet seedlings

In general, the application of filter mud significantly promoted sugar beet growth, and with the increase of applying filter mud, the biomass of sugar beet seedlings also increased gradually (Figure 1). Therefore, the relevant indexes of sugar beet seedlings' growth were determined as verification. When the proportion of filter mud was 5% and 7%, the plant height of sugar beet seedlings was 16.633 cm, which was the maximum of the experiment. The plant height, leaf length, leaf width, leaf area, and fresh and dry weight of above-ground parts of a single plant reached maximum values in the whole plant when the proportion of filter mud was 7%, increasing by 51%, 52%, 42%, 135%, 88%, and 106% compared to the control, respectively (Table 2). The result showed that applying filter mud promoted plant biomass production. Leaf morphological features, such as leaf length, leaf width and leaf area determine the quantity of photosynthetic capacity (Mathan et al., 2016).

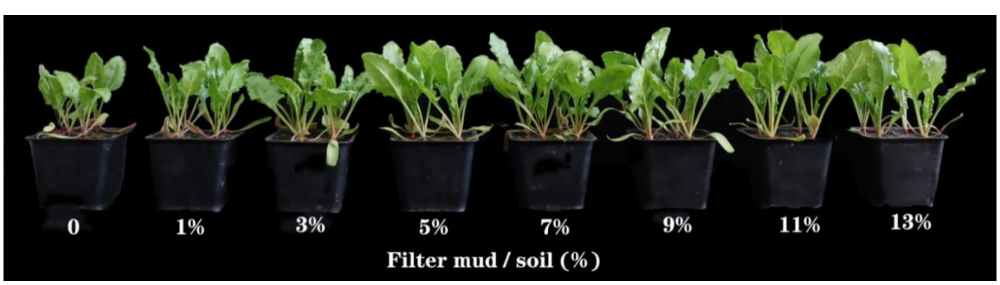

Figure 1. Effect in sugar beet plants under control (non-application of filter mud) and different proportions of filter mud/soil.

Table 2. The growth of sugar beets in the case of different proportions of mud filtration. Treatments were respectively non-application of filter mud, and seven proportions of filter mud and neutral black soil (1%, 3%, 5%, 7%, 9%, 11%, and 13%). Values are the mean of three replicates ± standard error. Different letters within the same column indicate significant differences between treatments ($P < 0.05$).

Treatment: Filter mud/soil	Plant height	Leaf length	Leaf width	Leaf area	Fresh weight of single plant in above-ground part	Dry weight of single plant in above-ground part
%	cm	cm	cm	cm^2	g	g
0	11.0333 ± 0.6429c	6.6667 ± 0.6429c	3.7000 ± 0.2646c	48.0933 ± 0.2701f	2.9867 ± 0.2836c	0.2767 ± 0.0379c
1	11.6333 ± 0.4041c	7.1000 ± 0.6000c	3.7667 ± 0.2517c	55.4467 ± 0.3937e	3.5033 ± 0.0850b	0.3200 ± 0.0100c
3	14.6333 ± 1.0116b	8.7333 ± 1.1015b	4.5000 ± 0.5000b	72.8800 ± 1.8220d	3.7850 ± 0.3650b	0.4350 ± 0.0050b
5	16.6333 ± 0.2082a	10.1333 ± 0.1528a	5.2000 ± 0.6083a	103.8217 ± 0.2323c	5.5350 ± 0.2950a	0.5500 ± 0.0500a
7	16.6333 ± 0.7767a	10.1667 ± 0.5033a	5.2667 ± 0.3215a	112.9883 ± 0.7666a	5.6050 ± 0.3250a	0.5700 ± 0.0600a
9	15.6667 ± 0.6110ab	9.9667 ± 0.4619a	5.1667 ± 0.0577a	112.3400 ± 1.2278a	5.4300 ± 0.1700a	0.5150 ± 0.0250a
11	15.6333 ± 0.2517ab	9.9333 ± 0.5132a	5.2333 ± 0.1528a	111.6600 ± 0.4612a	5.3250 ± 0.3650a	0.5000 ± 0.0624ab
13	15.7000 ± 0.6557ab	10.0667 ± 0.4163a	5.2333 ± 0.2517a	109.4233 ± 1.7375b	5.5067 ± 0.0709a	0.5233 ± 0.0306a

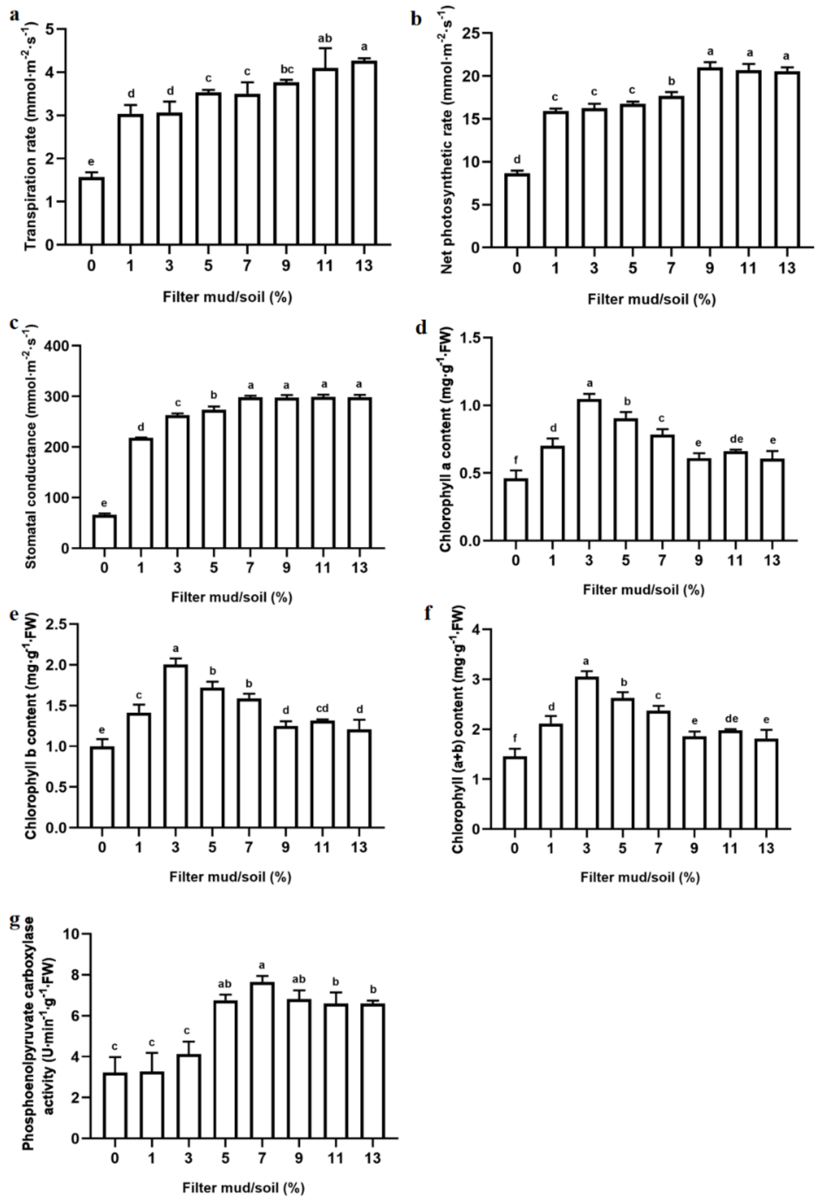

Figure 2. Effects of applying eight different proportions of filter mud on photosynthesis-related parameters in leaves of sugar beet. Transpiration rate (a), net photosynthetic rate (b), stomatal conductance (c), chlorophyll *a* content (d), chlorophyll *b* content (e) and chlorophyll (*a*+*b*) contents (f) of sugar beet plants, phosphoenolpyruvate carboxylase activity (g).

Effect on photosynthetic pigments content and photosynthetic parameters of sugar beet seedlings

Plant growth and productivity are generally controlled by photosynthetic pigments (Carbonell et al., 2000). Improved photosynthesis results in higher biomass accumulation and total crop yield (Sarraf et al., 2021). Efficient photosynthesis leads to higher biomass accumulation, which mainly determines the overall performance of plants (Long et al., 2006). Hence, chlorophyll *a*, chlorophyll *b*, chlorophyll (*a*+*b*) content, and some photosynthetic parameters including transpiration rate, net photosynthetic rate, stomatal conductance, and PEPC activity of beet leaves were measured. The application of filter mud significantly affected the photosynthetic parameters and photosynthetic pigment content of sugar beet seedlings. At the point where the percentage of filter mud changed from 0% to 3%, all photosynthetic pigment contents of sugar beet seedlings in this experiment showed a similar upward trend and increased significantly. When the proportion of filter mud was 3%, chlorophyll *a*, chlorophyll *b*, and chlorophyll (*a*+*b*) content reached the highest which increased respectively by 127%, 101%, and 109% compared with the control (Figure 2).

With the application of filter mud, the transpiration rate of sugar beet leaves gradually increased and reached the highest when the proportion of filter mud was 13%, which increased by 172% compared with the control (Figure 2a). When the proportion of filter mud was 9%, the net photosynthetic rate reached the highest value, which increased by 142% compared with the control (Figure 2b). But there was no difference in net photosynthetic rate where the percentage of filter mud changed from 9% to 13%. When the proportion of filter mud was 7%, the stomatal conductance of sugar beet leaves increased by 350% compared with the control (Figure 2c). However, there was no difference in stomatal conductance where the percentage of filter mud changed from 7% to 13%. The degree of stomatal opening affects plant photosynthesis and transpiration and is closely related to the plant's absorption and utilization of atmospheric CO_2 and the degree of transpiration water loss (Luo et al., 2013). The plant canopy influences the surface water and energy balance by controlling water vapor and CO_2 of leaf surfaces through stomata or stomatal conductance (Miner et al., 2017). The stomatal conductance of sugar beet seedling leaves did not change significantly in several treatments with the percentage of filter mud exceeding 7%, and the net photosynthetic rate and transpiration rate of sugar beet seedlings were also limited due to the limitation of stomatal opening. This showed that filter mud had a certain promotion effect on the photosynthesis and transpiration of sugar beet seedlings, but this promotion effect was positively correlated with the growth of sugar beet within a certain range of filter mud content, beyond which there was nonsignificant change in the promotion effect on sugar beet growth, but nonsignificant inhibition phenomenon appeared either.

The enhancement of photosynthesis was mainly characterized by the enhancement of the activity of the photosynthesis enzyme and the enhancement of CO_2 assimilation (Zivcak et al., 2013; Urban et al., 2017). Similarly, when the proportion of filter mud was 7%, the PEPC activity of sugar beet leaves increased by 138% compared with the control (Figure 2g). Therefore, when the proportion of filter mud was 7%, the values of stomatal conductance and PEPC activity reached the maximum value, which was consistent with the proportion of filter mud when the biomass of sugar beet leaves reached the maximum value. And then decreased slowly when the sludge proportion reached 7%. These results indicated that increases in photosynthetic pigment content, as well as photosynthetic parameters, contribute to the growth of sugar beet biomass. Therefore, it was hypothesized that the application of filter mud could effectively increase the PEPC activity of sugar beet, resulting in faster photosynthesis and increased accumulation of photosynthetic products.

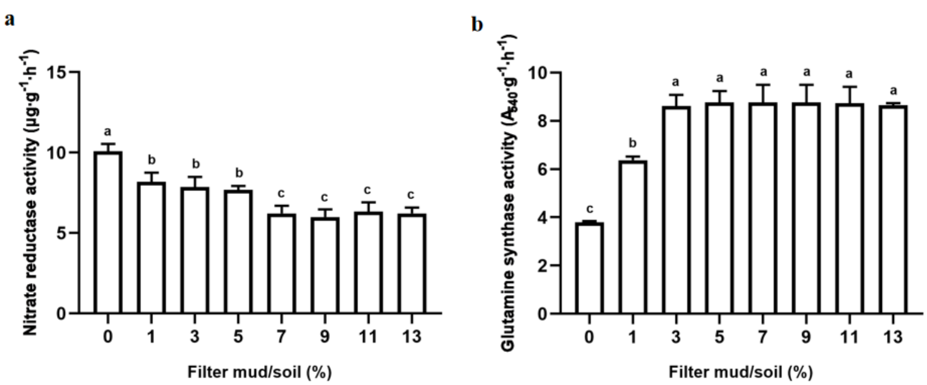

Figure 3. Effects of applying eight different proportions of filter mud on nitrate reductase activity (a) and glutamine synthase activity (b) in leaves of sugar beet.

Effect on nitrate reductase and glutamine synthetase activities of sugar beet seedlings

Nitrate reductase (NR) is one of the key enzymes of N assimilation in plants (Chamizo-Ampudia et al., 2017; Fu et al., 2020), it plays a key role in the uptake and utilization of N fertilizer by crops. Nitrate reductase activity in plants directly affects the utilization of soil inorganic N, which in turn affects the yield and quality of crops. To verify the effect of NR and GS activities on the N content of sugar beet, NR, and GS activities of sugar beet seedling leaves were measured. Filter mud had a significant effect on NR and GS activities in sugar beet leaves. The NR activity of seedlings of the control group remained at a high level. The activity of NR started to decrease at 1% filter mud. The activity of NR decreased again at 7% of the applied filter mud (Figure 3a). After the application of filter mud, the NR activity of sugar beet leaves gradually decreased (Figure 4a), the converted N content became less, the protein content was less, N content in the plant naturally decreased (Figure 3a). However, it has been shown that the presence of NH_4^+-N in the environment in turn affects the uptake of NO_3^--N by plants (Wang et al., 2007). Therefore, the ratio of NH_4^+-N to NO_3^--N affects the NR activity in plants. In a study, it was shown that NR increased with the proportion of nitrate N and decreased with the proportion of ammonium N (Li et al., 2003). The NR activity of seedlings was negatively correlated with the growth of sugar beet seedlings in this experiment, and further experiments are needed to verify the reason. Glutamine synthase (GS) is one of the enzymes needed for N metabolism in plants. Significant increase in GS activity when the concentration of filter mud was 3% (Figure 3b). The GS activity was positively correlated with the growth of sugar beet seedlings.

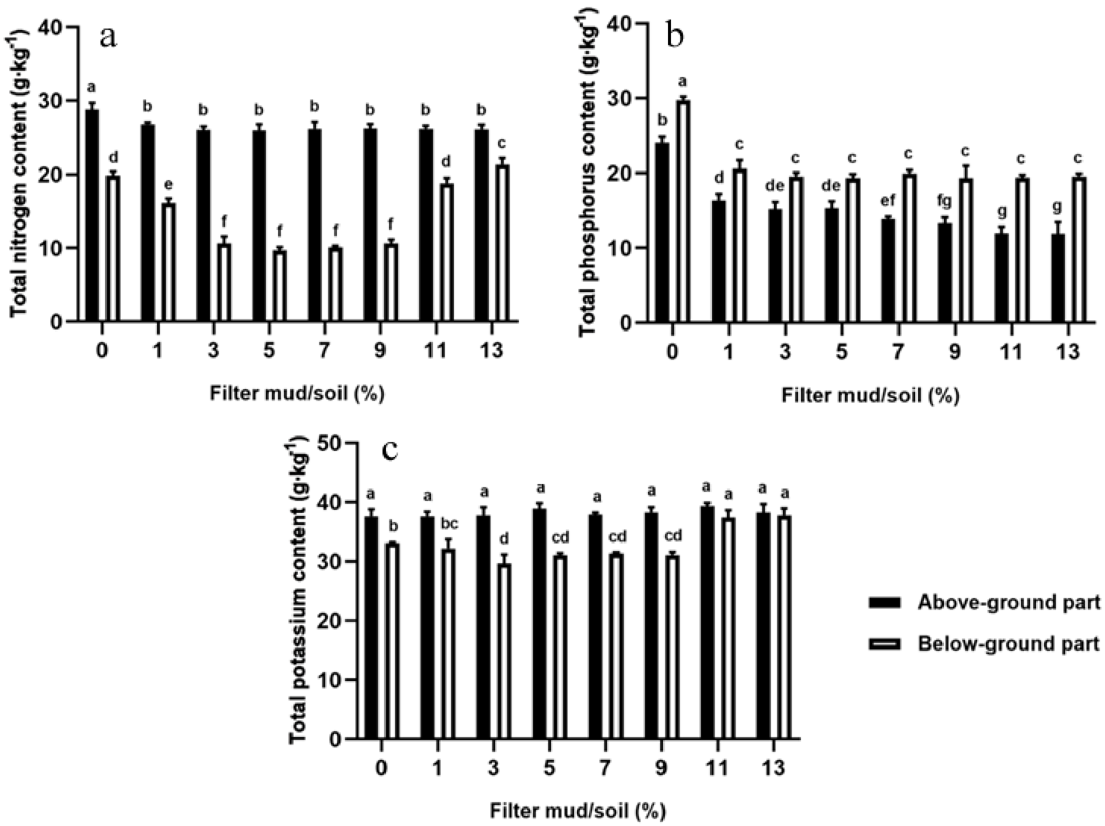

Figure 4. Effects of applying eight different proportions of filter mud on nutrients parameters in the above-ground parts and below-ground parts of sugar beet. Total N content (a), total P content (b), and total K content (c) of sugar beet plants.

Effect on nutrients parameters of sugar beet seedlings

To verify that the nutrients contained in the filter mud are absorbed by the sugar beet, we measured the nutrients in the sugar beet above-ground parts and below-ground parts. The application of filter mud had a significant effect on N and P content in above-ground parts and below-ground parts of sugar beet seedlings. On the contrary, the application of filter mud had a nonsignificant effect on K content in above-ground parts of sugar beet seedlings, but significant effects on sugar beet below-ground parts. The N content of the seedlings above-ground parts of the control group remained at a high level. All sugar beet seedlings have a lower N content in the below-

Table 3. Effect of different proportions of filter mud on physicochemical properties of soil planted with sugar beets. Treatments were non-application of filter mud, and seven proportions of filter mud and neutral black soil (1%, 3%, 5%, 7%, 9%, 11% and 13%). Values are the mean of three replicates ± standard error. Different letters within the same column indicate significant differences between treatments ($P < 0.05$).

Filter mud/soil	Inorganic N	Available P	Available K	Organic matter	pH
%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	%	
0	48.7200 ± 1.6800g	51.7341 ± 1.4451g	212.3344 ± 1.2054d	2.6249 ± 0.0225e	7.3667 ± 0.0416c
1	54.5067 ± 1.7108f	58.9595 ± 3.8233f	213.2622 ± 0.4019cd	2.6399 ± 0.0260e	8.1467 ± 0.0115b
3	59.8267 ± 2.6612e	66.6667 ± 1.6686e	216.5131 ± 3.1935bcd	2.6700 ± 0.0225de	8.1767 ± 0.0208b
5	62.2533 ± 1.3812de	76.3006 ± 5.2103d	218.3727 ± 4.5358bc	2.7226 ± 0.0130cd	8.3100 ± 0.0265a
7	64.9600 ± 2.0191cd	84.4894 ± 3.3373c	219.7657 ± 2.1311b	2.7601 ± 0.0390c	8.3067 ± 0.0153a
9	67.2000 ± 0.5600c	91.2331 ± 3.6367bc	219.0695 ± 3.9662b	2.8352 ± 0.0344b	8.3467 ± 0.0252a
11	70.9333 ± 0.8554b	96.0501 ± 5.0750ab	220.9286 ± 2.6410ab	2.8803 ± 0.0130b	8.3200 ± 0.0173a
13	76.3467 ± 1.7108a	101.3487 ± 5.8402a	225.1179 ± 2.8233a	2.9704 ± 0.0853a	8.3500 ± 0.0557a

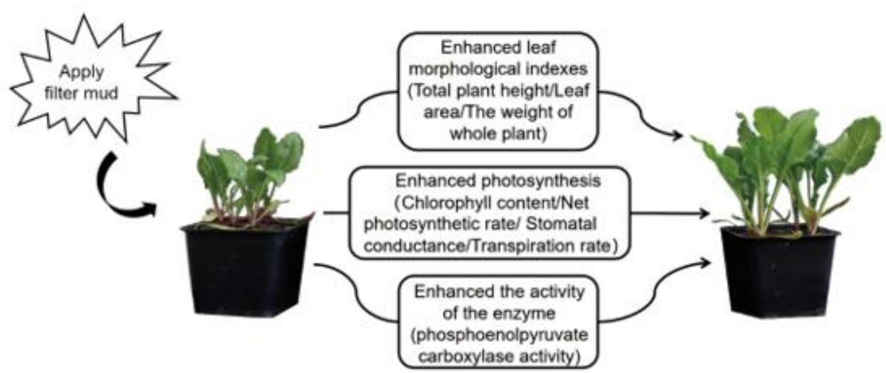

Figure 5. Schematic diagram summarizing the response of sugar beet plants to filter mud application.

ground parts than in the above-ground parts. After adding filter mud, the N content of seedlings above-ground parts in all treatment groups was significantly lower than in the control group. At the point where the percentage of filter mud changed from 3% to 9%, the N content of seedlings below-ground parts reached the lowest (Figure 4a). In related studies, it was shown that appropriate N nutrition could promote chlorophyll formation, enhance photosynthetic efficiency, and play a role in delaying leaf senescence and yellowing in terms of phenotype. However, excess N can lead to a nutrient imbalance in plants, hinder chlorophyll formation and reduce the photosynthetic rate (Zhao et al., 2020). This explains the decrease in chlorophyll ($a+b$) content when the inorganic N content of the below-ground part of sugar beet seedlings is higher. With the application of filter mud, the P content of the sugar beet above-ground part gradually decreased and reached the lowest when the proportion of filter mud was 13%, which decreased by 51% compared with the control (Figure 4b). All sugar beet seedlings had a higher P content in the below-ground parts than in the above-ground parts. The P content in the below-ground parts of all treatment groups was lower than in the control group. And there was no difference in the P content of beet below-ground parts between the treatment groups. When the proportion of filter mud was 13%, the K content of sugar beet below-ground parts reached the highest (Figure 4c).

Effect on physicochemical properties of the soil

Inorganic N, available P, available K, and organic matter content of the filter mud were significantly higher than those of the black soil; they were measured in the soil planted with sugar beet after adding filter mud. Application of filter mud affected the content of inorganic N, available P, available K, and organic matter planted with sugar beet in the soil significantly, and they increased gradually with the increase of the proportion of filter mud (Table 3). When the proportion of filter mud was 13%, compared with the control, the content of inorganic N increased by 57%, available P increased by 96%, available K increased by 6%, and organic matter increased by 13% (Table 3). After planting sugar beet, the inorganic N content of the soil gradually increased as the proportion of filter mud increased (Table 3). However, the trend in the N content of the plant differed from that of the soil. As the content of filter mud increased, the available P content of the soil increased, but the P content of the above-ground parts and below-ground parts of the plants did not increase (Table 3, Figure 3b). The trend in the K content of the plant also differed from that of the soil. The increase in soil inorganic N, available P, and available K content after planting sugar beet was due to two factors. On the one hand, it was due to a substantial increase in their content by the nutrient solution, on the other hand, it was due to a smaller amount taken up by sugar beet. Sugar beet is suitable for alkaline soil growth. When the proportion of filter mud was 7%, the soil tended to be weakly alkaline, which was the most suitable for the growth of sugar beet (Table 3). If the filter mud ratio is increased, the optimal pH range of beet may be exceeded, and the normal growth of microorganisms in the soil and the availability of some trace elements may be affected, thus causing soil environmental problems.

Based on our results, a schematic diagram summarizing the response of sugar beet to applying filter mud was presented (Figure 5). The results showed that the effects of filter mud on the growth and photosynthesis of sugar beet seedlings in neutral black soil were complex and multifaceted. Future studies will focus on the molecular mechanism of filter mud promoting the growth of sugar beet seedlings.

CONCLUSIONS

In summary, when the proportion of filter mud was more than 7%, leaf biomass of sugar beet seedlings remained stable. Certain range of filter mud could promote the growth of sugar beet seedlings. This result implied that 7% of filtered mud maintains the critical value of maximum biomass of sugar beet seedlings. The growth of sugar beet biomass was accompanied by changes in photosynthetic pigment content, photosynthetic parameters, and N metabolizing enzymes in sugar beet seedlings. The application of filter mud could increase the content of inorganic N, available P, available K, and organic matter and improve the physical and chemical properties of the soil, which could relate to the growth of sugar beet seedlings. The effect of filter mud on the growth state and physiological changes of sugar beet is therefore a complex process determined by several physiological metabolic pathways. This provides a reference for the application of filter mud to sugar beet production in the future.

Author contributions

Conceptualization: J.W., G.G., Y.W. Methodology: J.W. Software: J.W., G.F. Validation: H.C. Formal analysis: J.W. Investigation: J.W. Resources: G.G., Y.W. Data curation: H.C. Writing-original draft: J.W. Writing-review & editing: Y.W. Visualization: L.W. Supervision: Y.W. Project administration: L.Y. Funding acquisition: G.G., Y.W. All co-authors reviewed the final version and approved the manuscript before submission.

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