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RESEARCH ARTICLE

Variations in organic carbon, aggregation, and enzyme activities of gangue-fly ash-reconstructed soils with sludge and arbuscular mycorrhizal fungi during 6-year reclamation

Ningning Yin [1] · Zhen Zhang [2] · Liping Wang [1] · Kuimei Qian [3]

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Abstract Mining activities can cause drastic disturbances in soil properties, which adversely affect the nutrient cycling and soil environment. As a result, many efforts have been made to explore suitable reclamation strategies that can be applied to accelerate ecology restoration. In this study, we reconstructed mine soils with fly ash, gangue, sludge, planted ryegrass, and inoculated arbuscular mycorrhizal fungi (AMF) in Pangzhuang mine of Xuzhou during 2009 to 2015. The soil aggregation process, enzyme activities (i.e., invertase, urease and acid phosphatase activities), soil organic carbon (SOC) as well as other soil nutrients such as nitrogen, phosphorus, and potassium contents of the reconstructed mine soils were monitored during 6-year reclamation. The integrated application of sludge and AMF led to a promising reclamation performance of mining areas, in which soil aggregate stability, enzyme activities, SOC, and ryegrass biomass were effectively enhanced. The micro-aggregates (< 0.25 mm) decreased with the increase of macro-aggregates (> 0.25 mm) during the reclamation, indicating that macro-aggregates were gradually formed from micro-aggregates during the pedogenesis of reconstructed mine soils. The correlation analysis shows that SOC contents in aggregate fraction of 0.25~0.5 mm were correlated with aggregate distribution and enzyme activities. Enzyme activities, however, were not significantly correlated with aggregate distribution. The outcomes from the present study could enrich our understanding on soil property changes in pedogenesis process of reconstructed mine soils, and meanwhile, the employment of sludge combined with AMF is suggested to be an effective alternative for the mine soil reclamation.

Keywords Arbuscular mycorrhizal fungi (AMF) inoculation · Reconstructed mine soils reclamation · Soil organic carbon (SOC) · Soil aggregation · Sludge addition

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Ningning Yin and Zhen Zhang contributed equally to this work and should be considered co-first authors.

✉ Liping Wang
wlpcumt@126.com

[1] School of Environment and Spatial Informatics, China University of Mining & Technology, No. 1, Daxue Road, Xuzhou, Jiangsu 221116, China

[2] Department of Civil and Environmental Engineering, The Hong Kong University of Science and Technology, New Territories, Hong Kong

[3] School of Environmental Engineering, Xuzhou Institute of Technology, Xuzhou, China

Introduction

In recent decades, an increasing number of mining activities have caused serious damages to the soils, drastically disturbing the soil properties, and adversely affecting the nutrient cycling (Anderson et al. 2004; Burke et al. 2014). Zhang et al. (2015) reported that the modern mining technology can accelerate runoff and soil erosion by adversely affecting soils and subsurface geological structures. In addition, there is a significant loss of soil organic carbon (SOC) and associated nutrients (e.g., nitrogen) for the mine soils (Wang et al. 2015; Jacinthe and Lal 2007; Chaudhuri et al. 2013). Generally, the mine soils are often found to be of high bulk density (Chaudhuri et al. 2013), low pH (Zornoza et al. 2010), low nutrient availability (Juwarkar et al. 2010), and poor soil structure (Sheoran et al. 2010), which restrict plant growing. Thus, there is a need to reclaim the disturbed mine soils in order to accelerate ecology restoration.

It has been found that adding organic amendments (e.g., coal combustion byproducts, biosolids, biochar, swine or poultry manure, sewage or papermill sludge, sawdust or wood residue, limestone slurry byproduct) to soil can enhance soil fertility and accelerate ecosystem recovery (Bendfeldt et al. 2001; Moon et al. 2016; Ouyang et al. 2016; Wang et al. 2008; Zhang et al. 2013). Of all the reported organic amendments, the application of sludge has been proofed to be capable of decreasing the soil bulk density (Juwarkar et al. 2010; Asensio et al. 2014), increasing the SOC (Méndez et al. 2012) and biomass productivity (Juwarkar et al. 2010; Moreno-Penaranda et al. 2004), as well as improving the aggregate stability (Bendfeldt et al. 2001). Apart from the organic amendment, arbuscular mycorrhizal fungi (AMF) inoculation can promote the transfer of nutrients from soil to host plant, consequently leading to the enhancement of the plant growth (Kohler et al. 2015). Also, previous studies have also indicated that AMF produces a glycoprotein, glomalin that acts as an insoluble glue to stabilize aggregates (Wright and Anderson 2000). The AMF hyphae have been found to be one of the most important components in biotic influences on soil aggregation (Jastrow et al. 1998). As abovementioned, sludge and AMF play different roles in improving the property of the mine soils. Thus, the integrated application of both sludge and AMF inoculation is expected to obtain a better performance of reclamation. However, to our best knowledge, the attempt of using sludge together with AMF for reclamation of mine soils is very limited (Curaqueo et al. 2014; Kohler et al. 2015). Therefore, evaluating the feasibility of such integrated approach for the reclamation of reconstructed mine soils is needed.

The soil aggregate stability is a frequently used indicator of soil property in the field of grassland, forest and agroecosystems (Bronick and Lal 2005; Nichols and Toro 2011; Six et al. 2000). The stability of soil aggregates influences a wide range of soil properties, such as carbon stabilization, soil porosity, water infiltration, aeration, compactness, water retention, hydraulic conductivity, resistance to erosion by water and overland flow (An et al. 2010). However, few studies have been performed to investigate the stability of the aggregate in the mine soils during the reclamation. This is likely because very few aggregates were found in the initial stage of reconstructed mine soils until the aggregation process proceeds to a relatively long time (Sheoran et al. 2010). Aggregation process of the reconstructed mine soils is crucial for soil property enhancement, but the changes are not clear, particularly in the initial phases of pedogenesis for reconstructed mine soils. Thus, the changes of the aggregate stability in the mine soils over reclamation age deserve more research attention. Several studies have reported that there exists some relationship among aggregate, SOC, and enzyme activities in forest, grassland, and agroecosystems (Ayoubi et al. 2012; Liu et al. 2013; O'Brien and Jastrow 2013; Wei et al. 2013). However, so far, few studies addressed SOC and enzyme activities involved in soil aggregation in reclaimed mine soils. Nevertheless, investigating the interaction between these parameters is expected to help us to better understand the changes of soil property during the mine soil reclamation and eventually obtain a more comprehensive evaluation on the reclamation performance.

Therefore, the objectives of this study were to (1) study the feasibility of integrated application of sludge and AMF on reconstructed mine soils during the reclamation, (2) investigate the role of aggregation process in the pedogenesis of soils with sludge addition and AMF inoculation, and (3) explore the correlation between aggregate stability, SOC, and enzyme activities in the reconstructed mine soils added sludge and inoculated AMF.

Materials and methods

Experimental design

The reconstructed substrates were fly ash, gangue, and sludge, which were collected from Pangzhuang Mine, power station of Pangzhuang mine and Yaozhuang waste water treatment plant, respectively. The reconstructed mine soils were made up of these substrates at different ratios (Table 1). SOC, total nitrogen (TN), and total phosphorus (TP) contents of the different substrates tested are given in Table 2. The local soil was a silty loam (Haplic Ustarents) in the USDA system according to Soil Survey Staff 2010. The field trial was carried out in Xuzhou Pangzhuang Mine. There are four plots (in triplicate) made of different reconstructed mine soils in the field (Fig. 1). The area of each plot was 5 m^2. A 20-cm stone dam was placed between each plot. The depth of the complex substrate was 15 cm. The annual average precipitation was 860 mm and annual mean temperature was 14 °C. The trial was carried out from 2009 to 2015. We broadcasted the ryegrass (*Loliumperenne*) seeds with 20~30 g m^{-2} in September of 2009 and March of 2013, respectively. The ryegrass was cut after 12-month growth, and the biomass were measured by the dry weight of shoot and root. No pesticides and fertilizers were treated to the soil while the ryegrasses grow. The reclamation of reconstructed mine soils are similar to initial phases of pedogenesis (Bodlák et al. 2012).

Soil sampling and physico-chemical analysis

Soil sampling was carried in September of 2009, October of 2013, and May of 2015 following a snake-shaped pattern in each plot respectively. Triplicate soil samples were collected at the depth of 0~15 cm and thoroughly mixed together. Thus, a total of 18 samples were obtained each time. Soil was then air-dried at room temperature.

Table 1 The characteristic of reconstructed mine soils

Reconstructed mine soils	Reconstructed substrates	Ratio	Inoculation
FG	Fly ash:gangue	5:95	Non-inoculation
FGS	Fly ash:gangue:sludge	10:60:30	
FG+	Fly ash:gangue	5:95	Glomus mosseae (G. m)[a]
FGS+	Fly ash:gangue:sludge	10:60:30	

[a] Inoculation density was 1 kg m^{-2}. *G. m* was one kind of AMF and provided by Mycorrhizal Biological Pilot of Qingdao Agriculture University (Qian et al. 2012)

The SOC was determined using the exothermic heating and oxidation with potassium dichromate method (GB7857-87, China). TN, TP, and total potassium (TK) concentrations of soils were determined using the method of Lu (1999).

Separation of soil aggregates

The size distribution of soil aggregate was determined by the Soil Aggregate Analyzer (DM200, China) according to the method of Nichols and Toro (2011). We select 100 g of air-dried soil. Four water-stable aggregate fractions with different sizes of > 2 mm, 2~0.5 mm, 0.5~0.25 mm, and < 0.25 mm were obtained using various sieves. Different fractions collected from various sieves were weighed and stored.

The mean weight diameter (MWD) of each sample was calculated as follows:

$$\mathrm{MWD} = \sum_{1}^{n+1} \frac{r_{i-1} + r_i}{2} \times m_i \qquad (1)$$

Where r_i denotes the mean diameter of the aggregates class, m_i is the percentage of the corresponding aggregates class weight in the total aggregates weight.

Soil enzyme activity analysis

In the present study, the activities of five types of soil enzymes were determined including invertase, dehydrogenase, urease, acid and alkaline phosphate. The selection of these five soil enzymes is because they are commonly involved in soil nutrient cycles, such as carbon, nitrogen and phosphorus cycle (Nannipieri et al. 2002; Kohler et al. 2015). The activities of soil invertase, dehydrogenase, urease, acid and alkaline phosphate were measured using the method of Liu et al. (2013).

Table 2 Characteristics of the reconstructed substrates

Components of complex substrate	SOC (g kg^{-1})	TN (g kg^{-1})	TP (mg kg^{-1})
Soil in coal mine	17.46	1.23	30.15
Coal gangue (G)	13.60	1.39	29.81
Fly ash (F)	0.77	0.41	31.27
Sludge (S)	47.67	17.69	298.46

The characteristics of the reconstructed substrates were referred to Qian et al. (2012)

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS software (Statistical Product and Service Solution, version 17.0). Significance was accepted at $p < 0.05$ in all cases. Linear regressions and coefficients of determination (r) were used to describe the relationship between aggregate and SOC as well as enzyme activities.

Results and discussion

Applicability of sludge addition and AMF inoculation on reconstructed mined soil reclamation

Soil enzyme activities, which can be used for estimating the soil biochemical and microbial activities, and, to some extent, could reflect the biodiversity of soils (Finkenbein et al. 2013; Liu et al. 2013). Meanwhile, the soil fertilizer level could be reflected by the content of three major macronutrients including nitrogen, phosphorus, and potassium (Sheoran et al. 2010). Organic matter is the major source of nutrients. These nutrients are helpful to get high plant biomass which is the main component of soil productivity (Mueller et al. 2010; Duan et al. 2015). In addition, soil aggregate was an important component of soil structure,which controls soil hydrology (Sheoran et al. 2010). All in all, the soil enzyme activities, nutrient status, plant biomass, and soil aggregate can be used as important indicators for reflecting the soil property changes in pedogenesis process of reconstructed mine soils.

As seen in Table 3, invertase, urease, dehydrogenase, acid and alkaline phosphatase activities in FGS (i.e., soils reconstructed with fly ash and gangue, and with sludge addition) were generally higher than that in FG (i.e., soils reconstructed only with fly ash and gangue). The enhancement on the investigated soil enzyme activities was due to the addition of sludge in FGS, since high nutrient content can be provided by the sludge (Shrestha and Lal 2006). Besides, the content of SOC in FGS (i.e., 18.3 g kg^{-1}) was higher than that in FG (i.e., 15.52 g kg^{-1}) in 2009 (Table 4), which showed that sludge

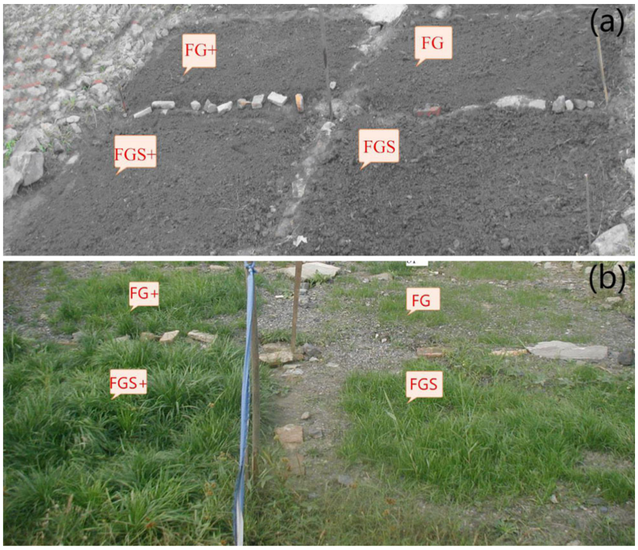

Fig. 1 The plots of field test on restoration of coal mine soil by mycorrhizal technology. **a** The original status of reconstructed mine soils before ryegrass planting in 2009; **b** the reconstructed mine soils after ryegrass planting in 2013. *FG* refers to soils only reconstructed with fly ash and gangue, *FG*+ refers to the soils reconstructed with fly ash and gangue and inoculated with AMF, *FGS* refers to soils reconstructed with fly ash, gangue and sludge, and *FGS*+ refers to soils reconstructed with fly ash, gangue, and sludge and inoculated with AMF

addition can effectively increase SOC content in soil. With the reclamation age prolonging to 2013 and 2015, the increase of SOC content in FGS was even more significant as compared with that in FG. This suggests that the addition of sludge plays an important role in promoting carbon sequestration by enhancing plant growth and development (Juwarkar et al. 2010). The speculation can be supported by the growing status of ryegrass in Fig. 1b. The TN, TP, and TK contents in FGS were also much higher than that in FG (Table 4). The above results are consistent with the previous study which reported that the variations of soil enzyme activities were closely correlated with soil nutrients cycles, such as carbon, nitrogen, and phosphorus (Ciarkowska et al. 2014; Finkenbein et al. 2013; Xian et al. 2015; Xu et al. 2015). Therefore, it can be concluded that the sludge addition is capable of restoring the damaged soils by effectively promoting the soil enzyme activities as well as the soil nutrients cycles. The role of sludge has been previously reported by Sheoran et al. (2010), which also showed that sludge can effectively enhance the soil enzyme activities and improve the soil nutrients status. It is interesting that, for all the groups themselves, the TN, TP, and TK contents were lower in 2013 and 2015 if compared with that in 2009 (Table 4). This may be due to the fact that more nutrients transferred from soil to plants by AMF hyphae. Several studies have reported that AMF inoculation has a positive effect on plant growth by enhancing nutrient and water uptake (Bouwmeester et al. 2007; Yamawaki et al. 2013). Moreover, it has been shown that particular plant–AMF combinations are efficient in stimulating plant growth and nutrient acquisition (Van der Heijden et al. 2006). And the growth status in FG+ and FG may support this speculation (Fig. 1b).

Unlike the enhancement of sludge on enzyme activities, the activities of invertase, urease, acid and alkaline phosphatase in FG+ (i.e., soils reconstructed with fly ash and gangue, and with AMF inoculation) were only slightly higher than that in FG (Table 3). As for the SOC content, it was almost in the same level between FG+ and FG in both 2013 and 2015, which suggests that the enhancing effect of AMF inoculation on SOC is not as obvious as the addition of sludge. AMF inoculation has no obvious effect on the SOC changes (Table 4). But the decreases of TK and TP in FG+ from 2009 to 2015 (i.e., 6.88 and

Table 3 Soils enzyme activities of reconstructed min soils

	Invertase (mg glucose g^{-1} day^{-1})	Urease (mg NH_3–N g^{-1} day^{-1})	Dehydrogenase (mg TPF g^{-1} day^{-1})	Acid phosphatase (mg phenol g^{-1} h^{-1})	Alkaline phosphatase (mg phenol g^{-1} h^{-1})
FG					
2009	13.03 (1.22) g	0.16 (0.02) g	0.26 (0.04) de	0.53 (0.05) i	0.29 (0.05) i
2013	30.55 (3.28) e	0.17 (0.09) g	0.21 (0.05) e	0.97 (0.06) h	0.42 (0.02) g
2015	31.45 (1.39) e	0.28 (0.12) de	0.22 (0.02) e	1.27 (0,05) fg	0.56 (0.03) e
FG+					
p;2009	19.21 (2.76) f	0.21 (0.03) fg	0.31 (0.05) bc	0.59 (0.07) i	0.48 (0.07) j
2013	29.76 (1.49) e	0.29 (0.03) ef	0.14 (0.02) f	1.01 (0.02) gh	0.59 (0.03) e
2015	32.78 (1.26) e	0.34 (0.02) cde	0.15 (0.01) f	1.45 (0.01) ef	0.65 (0.02) d
FGS					
2009	59.64 (8.05) d	0.38 (0.08) cd	0.31 (0.03) bc	1.6 (0.20) e	0.37 (0.05) h
2013	130.69 (5.34) b	0.40 (0.07) bc	0.22 (0.04) f	2.17 (0.06) d	0.54 (0.01) f
2015	132.57 (4.92) a	0.55 (0.02) a	0.21 (0.01) f	3.28 (0.02) a	0.78 (0.02) c
FGS+					
2009	99.3 (12.26) c	0.35 (0.09) cde	0.38 (0.04) ab	1.91 (0.22) d	0.69 (0.10) d
2013	133.1 (5.26) a	0.46 (0.11) b	0.43 (0.06) a	2.52 (0.03) c	0.95 (0.05) b
2015	135.6 (6.37) a	0.58 (0.12) a	0.39 (0.05) a	3.39 (0.04) a	1.27 (0.08) a

Dissimilar letters indicate significant differences whereassimilar letters indicate no significant differences among the treatments in the same column at $p < 0.05$

Table 4 Soil nutrients and ryegrass biomass in reconstructed min soils

	SOC, g kg^{-1}	TN, g kg^{-1}	TP, mg kg^{-1}	TK, mg kg^{-1}	Ryegrass biomass, kg
FG					
2009	15.52 (2.34) f	1.15 (0.17) ef	26.1 (3.36) fg	4.41 (0.55) b	6.44 (0.87) e
2013	28.64 (1.08) d	1.26 (0.15) e	21.67 (1.92) fg	1.26 (0.28) hi	10.56 (1.35) d
2015	29.38 (1.03) d	1.29 (0.38) ef	20.48 (2.47) gh	1.28 (0.08) hi	[a]
FG+					
2009	16.08 (2.52) ef	1.11 (0.14) e	24.16 (3.02) f	4.80 (0.70) b	15.32 (2.07) c
2013	30.05 (2.07) bc	1.32 (0.11) e	17.75 (1.03) hi	1.32 (0.13) ghi	16.43 (2.15) c
2015	30.98 (2.38) c	1.22 (0.29) e	17.28 (2.92) hi	1.37 (0.08) ghi	[a]
FGS					
2009	18.3 (2.87) ef	4.04 (0.61) a	63.21 (7.9) b	5.97 (0.75) a	11.21 (1.40) d
2013	34.49 (1.65) bc	2.32 (0.23) c	39.52 (3.21) c	2.32 (0.27) f	16.82 (1.68) c
2015	35.16 (1.27) abc	1.67 (0.28) d	30.48 (2.83) e	2.92 (0.37) de	[a]
FGS+					
2009	19.40 (2.74) e	4.09 (0.55) a	67.68 (7.78) a	6.28 (0.85) a	18.90 (2.74) b
2013	37.67 (1.29) a	2.83 (0.18) b	42.39 (2.39) c	2.83 (0.30) ef	20.62 (2.85) a
2015	38.12 (1.36) a	2.64 (0.38) b	36.39 (1.38) d	2.48 (0.28) f	[a]

Dissimilar letters indicate significant differences whereas similar letters indicate no significant differences among the treatments in the same column at $p < 0.05$

The ryegrass biomass was the total weight of the plant after planting 12 months

[a] Refers to the data were not measured

3.43 g kg^{-1}, respectively) were higher than that in FG (i.e., 5.62 and 3.13 g kg^{-1}, respectively) in corresponding period (Table 4). This may be caused that more nutrient transferred from soil to plants by AMF hyphae. Several studies have reported that AMF inoculation has a positive effect on plant growth by enhancing nutrient and water uptake (Bouwmeester et al. 2007; Yamawaki et al. 2013). Moreover, it has been shown that particular plant-AMF combinations are efficient in stimulating plant growth and nutrient acquisition (Van der Heijden et al. 2006). And the growing status of ryegrass in FG+ and FG may support this speculation.

In FGS+ (i.e., soils reconstructed with fly ash and gangue, and with both sludge addition and AMF inoculation), the nutrient status (i.e., SOC, TN, TP, and TK contents), enzyme activities in 2015 were the highest (Tables 3, 4, and 5). The possible reasons include: (1) the sludge may improve soil nutrient status; (2) the sludge may stimulate mycorrhiza formation and soil microbial function, since the sludge can provide the soil microbe for carbon and energy; and (3) the introduced AMF may promote the transfer of nutrients from the reconstructed mine soil to the host plant, thus leading to an enhancement of plant growth (Kohler et al. 2015). The above results suggest that sludge addition and AMF inoculations are complementary to each other and the integrated application of them can be a promising alternative for the reclamation of reconstructed mine soils.

Aggregation process involved in soils with sludge addition and AMF inoculation during 6-year reclamation

The aggregation process involved in FGS+ was further analyzed and the aggregates distribution and stability can be seen in

Table 5 Distribution and stability of soil aggregates

	> 2 mm, %	0.5~2 mm, %	0.25~0.5 mm, %	< 0.25 mm, %	MWD[a], mm
FG					
2013	4.7 (0.41)	23 (0.83)	15 (0.48)	57 (0.97)	0.46 (0.01)
2015	11 (0.49)	48 (0.25)	22 (1.38)	17 (0.38)	0.82 (0.01)
FG+					
2013	5.4 (0.16)	24 (0.44)	17 (0.35)	53 (0.77)	0.48 (0.01)
2015	12 (0.15)	46 (0.32))	28 (0.90)	14 (0.76)	0.82 (0.01)
FGS					
2013	4.6 (1.04)	28 (0.78)	17 (0.19)	50 (0.89)	0.52 (0.01)
2015	12 (0.66)	47 (0.87)	25 (0.36)	16 (0.93)	0.82 (0.01)
FGS+					
2013	7.1 (0.22)	30 (0.33)	16 (0.85)	47 (1.18)	0.56 (0.01)
2015	12 (0.17)	48 (0.23)	25 (0.34)	14 (0.23)	0.84 (0.01)

[a] MWD refers to the mean weight diameter

Table 5. The aggregate fraction at the size of < 0.25 mm comprised the largest proportion of soil aggregates and accounted for 47 % of the total soil mass; whereas the fraction at the size of > 2 mm accounted for the smallest proportion (i.e., 7.1 % of the total soil mass) in 2013. In 2015, the predominant fraction of soil aggregates was at the size of 0.5~2 mm, which accounted for 42.92~48 %. The proportions of aggregate fractions at the sizes of > 2 mm, 0.5~2 mm 0.25~0.5 mm increased by 5.1, 18, and 9.2 % in the total soil while < 0.25 mm decreased by 33 %. This variation is expected as the reconstructed mine soils are not tilled and ryegrass planting increase root biomass, exudates, and litter inputs, thus, favors the aggregation of soil (Tisdall and Oades 1982; Qiu et al. 2012). Therefore, the results could imply macro-aggregates (> 0.25 mm) were coagulated from micro-aggregates (< 0.25 mm) with temporary and transient organic binding agents. The pedogenetic process in reconstructed mine soils can be described through the changes in the basic soil parameters. The changes of soil aggregates distribution improved the soil structure and facilitated pedogenetic of reconstructed mine soils.

Our results show that in the year of 2013, the proportion of macro-aggregates in FGS (50 % for the total proportion of aggregate fractions of > 2 mm, 0.5~2 mm 0.25~0.5 mm) was higher than that in FG (43 %). The aggregate stability (i.e., MWD) in FGS was also found to be higher than that in FG. The abovementioned results suggest that the organic matter involved in the sludge may serve as a nucleus for aggregate formation (Six et al. 1998) and meanwhile, it can also increase the aggregate cohesion (Bandyopadhyay et al. 2010; Yang et al. 2007). Meanwhile, it was found that the enhancing effect of AMF inoculation on the aggregation of the fraction of > 2 mm is significant in 2013. While the MWD in FG+ was higher than that in FG. All these results imply that AMF could promote the stability of macro-aggregate (Jastrow et al. 1998; Liu et al. 2013; Wright and Anderson 2000). Although it can be seen that the addition of sludge and AMF alone has no significant enhancing effect on the stability of aggregate especially in 2015, it is interesting that the MWD in FGS+ was much higher than FG. The reason is speculated as follows. It is reported that organic matter was the main biding agents during the process of aggregation and aggregate stability (Six et al. 2000). The organic matter involved in the sludge provides the main nucleus for aggregates. Meanwhile the AMF hyphae could wind the micro-aggregates to form macro-aggregate and lead to stabilization of the aggregates (Bronick and Lal 2005). Therefore, the integrated application of sludge and AMF can effectively improve the soil aggregate stability.

Correlation analysis among aggregate, SOC, and enzyme activities in soils added with sludge and inoculated with AMF

In order to better understand the changes of soil property during the soil reclamation and eventually obtain a more comprehensive evaluation on the reclamation performance, the interaction among aggregate distribution, SOC, and enzyme activities were investigated by further performing the correlation analysis among these parameters. SOC contents in different fractions of aggregates in FGS+ are shown in Fig. 2. SOC content was effectively increased by reclamation, and the increases varied with aggregate fractions. The content of SOC in aggregate fractions of > 2 mm, 0.5~2 mm, 0.25~0.5 mm, and < 0.25 mm increased by 1.51, 2.49, 2.91, and 2.73 g kg^{-1} from 2009 to 2015. The smallest increase occurred in the aggregate fraction of >2 mm, while the highest increase of SOC content were found in the fraction of < 0.25 mm in both 2013 and 2015 (i.e., 12.15 and 13.41 g kg^{-1}). The recovery of aggregation in reconstructed mine soils could be important for SOC accumulation. This is because the physical protection by soil aggregates is considered to be one of major mechanisms for protecting SOC from microbial attack and mineralization (Essington 2004; Ingram et al. 2005). And meanwhile, soil aggregate stability can be enhanced by this association (Six et al. 1998, 2000). The SOC and soil aggregates mutually protect each other. Our results showed high correlation coefficients ($r = 0.91$, $p = 0.01$ and $r = 0.90$, $p = 0.01$) between SOC and aggregate fraction (> 2 mm, 0.25~0.5 mm) (Table 6).

Reclamation enhanced acid phosphatase activities in different sizes of soil aggregate (Fig. 3a). Acid phosphatase activities were the highest in fraction of < 0.25 mm and the lowest in the fraction of > 2 mm in 2013. Acid phosphatase in the aggregate fraction of > 2 mm in 2015 was the highest with the increase of 15.31 % as compared

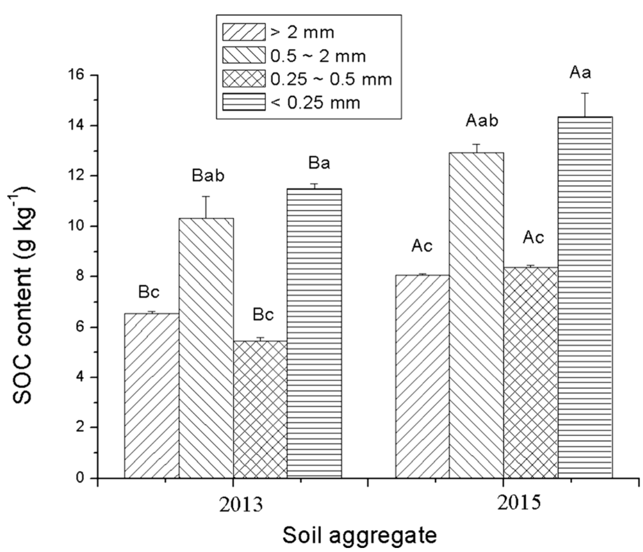

Fig. 2 SOC contents in different soil aggregate fractions of FGS+ in 2013 and 2015. *Error bars* represent standard errors of the means ($n = 3$). Significant differences are shown across four aggregate fractions with *lowercase letters* in the same year, *uppercase letters* indicate significant differences within one aggregate fraction between 2013 and 2015 ($p < 0.05$)

Table 6 Relationship between SOC content, enzyme activities (*Y*), and distribution of aggregate (*X*) in FGS soils (*n* = 12)

Soil property		Regression model	Correlation coefficient (*r*)	*p*
SOC	> 2 mm	*Y* = 13.8644 − 0.51055*X*	−0.91182[a]	0.01132
	05~2 mm	*Y* = 113.03842 − 0.00869*X*	−0.02249	0.96627 ns
	0.25~0.5 mm	*Y* = 43.27986 − 1.42233*X*	−0.90202[a]	0.01393
	< 0.25 mm	*Y* = 5.36877 + 0.46365*X*	0.53551	0.27352 ns
Invertase activity	> 2 mm	*Y* = 256.80973 − 18.83157*X*	−0.89118[a]	0.01712
	05~2 mm	*Y* = −74.17838 + 2.24374*X*	0.67796	0.13886 ns
	0.25~0.5 mm	*Y* = 64.00309 − 1.43271*X*	−0.83627	0.07755 ns
	< 0.25 mm	*Y* = 954.93305 − 49.07243*X*	−0.76597	0.07575 ns
Urease activity	> 2 mm	*Y* = 0.00373 + 0.0483*X*	0.5255	0.28431 ns
	05~2 mm	*Y* = 2.41054 − 0.04168*X*	−0.69073	0.12868 ns
	0.25~0.5 mm	*Y* = −1.08364 + 0.06999*X*	0.79397	0.0593 ns
	< 0.25 mm	*Y* = 2.76395 − 0.12706*X*	−0.75477	0.08283 ns
Acid phosphatase activity	> 2 mm	*Y* = 41.77825 − 3.20503*X*	−0.89077[a]	0.01725
	05~2 mm	*Y* = −75.23213 + 1.78902*X*	0.75742	0.08113 ns
	0.25~0.5 mm	*Y* = 8.89342 − 0.25311*X*	−0.91133[a]	0.01144
	< 0.25 mm	*Y* = −4.18496 + 0.47971*X*	0.75418	0.08321 ns

[a] Significant at $p < 0.05$

ns means not significant

with that in 2013. Urease activity is higher in 2015 as compared with that in 2013 (Fig. 3b). In both 2013 and 2015, the activities of urease were found to be higher in fraction of 0.25~0.5mm than that in other fractions. The invertase activities in aggregate fractions of > 2 mm, 0.5~2 mm, and 0.25~0.5 mm increased from 2013 to 2015 (Fig. 3c). Invertase activities were highest in fraction < 0.25 mm in 2013 and > 2 mm in 2015, respectively. The generally increase of the three enzyme activities in different aggregate fraction simply the stimulation of soil microbe with the aggregation during the reclamation. But the results obtained from the correlation analysis show that the relationship of enzyme activities and aggregate was not significant (Table 6). Some researchers also found that enzyme activities were distributed unequally among particle-sized soil fractions (Saviozzi et al. 2007; Qin et al. 2010). These unequal distributions of enzyme activities may be caused by the different soil nutrients availabilities and qualities (Wallenius et al. 2011). It was reported that higher SOC and TN contents were likely responsible for the higher enzyme activities observed in forest on Changbai Mountain (Xu et al. 2015). The correlation analysis showed that the activities of invertase, urease and acid phosphatase were significantly correlated with the SOC content of soil aggregates fraction of 0.25~0.5 mm ($r = 0.93$, -0.93, and 0.99 for invertase, urease, and acid phosphatase activity, respectively) (Table 7). The corn residue C was reported to accumulate linearly in the whole soil instead of in the fine fraction (Poirier et al. 2013). A positive and significant relationship between enzyme activities and C concentrations in size-classed soil particles were also found in some researchers (Onweremadu et al. 2007; Yang et al. 2007). The high SOC contents of aggregate fraction of < 0.25 mm may explain a high level of enzyme activity was involved in this fraction. According to Bodlák et al. (2012), the crucial role of SOC in the pedogenesis process makes it an appropriate tool for evaluating the soil property in a reclaimed post mining landscape. The application of

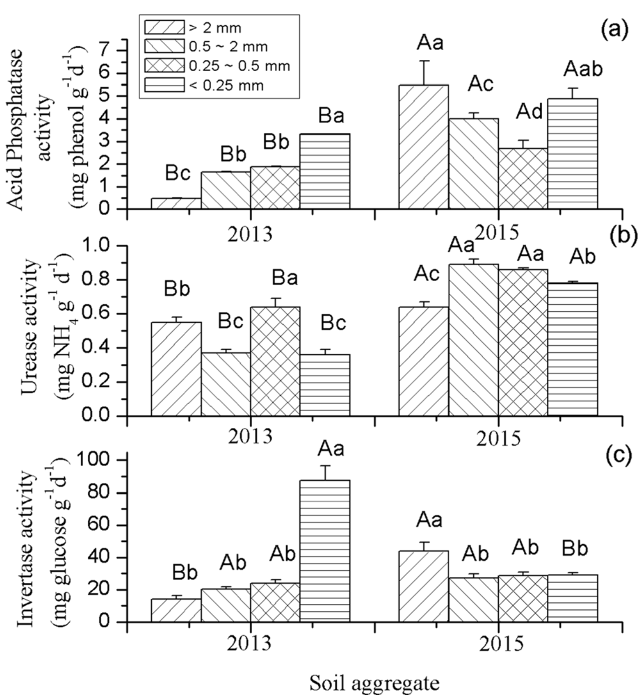

Fig. 3 Activities of **a** acid phosphatase, **b** urease, and **c** invertase in different sizes of FGS+ soil aggregate in 2013 and 2015. *Error bars* represent standard errors of the means ($n = 3$). Significant differences are shown across four aggregate fractions with *lowercase letters* in the same year, *uppercase letters* indicate significant differences within one aggregate fraction between 2013 and 2015 ($p < 0.05$)

Table 7 Relationship between enzyme activities (Y) and SOC content (X) in FGS+ soils ($n = 12$)

Soil property		Regression model	Correlation Coefficient (r)	p
Invertase activity	> 2 mm	$Y = -258.09515 + 37.3429X$	0.9895[a]	0.0002
	05~2 mm	$Y = -27.19788 + 4.03841X$	0.4747	0.34493 ns
	0.25~0.5 mm	$Y = 20.91505 + 0.87411X$	0.93186[a]	0.00681
	< 0.25 mm	$Y = 910.17932 - 59.98574X$	−0.81068	0.05037 ns
Urease activity	> 2 mm	$Y = 1.51149 - 0.12012X$	−0.73177	0.09827 ns
	05~2 mm	$Y = 2.88994 - 0.17906X$	−0.28971	0.57759 ns
	0.25~0.5 mm	$Y = 1.0626 - 0.05192X$	−0.92883[a]	0.00742
	< 0.25 mm	$Y = 2.74978 - 0.16293X$	−0.83796[a]	0.03726
Acid phosphatase activity	> 2 mm	$Y = -46.00238 + 6.37463X$	0.4677[a]	0.00461
	05~2 mm	$Y = -10.36437 + 1.04063X$	0.36451	0.47741 ns
	0.25~0.5 mm	$Y = 1.20374 + 0.17596X$	0.99897[a]	<0.0001
	< 0.25 mm	$Y = -3.41013 + 0.56116X$	0.76386	0.07706 ns

[a] Significant at $p < 0.05$

ns means not significant

sludge has been found to stimulate microbial growth (Green and Renault 2008; Sevilla-Perea and Mingorance 2015) and thus improve the enzyme production.

Conclusions

In this study, the integrated application of ryegrass planting, sludge addition and AMF inoculation for mine soils reclamation has been successfully demonstrated. After 6-year reclamation, soil property in the soils added with sludge and inoculated with AMF has been meliorated, as indicated by the increase of SOC content, soil aggregate stability and enzyme activities, as well as the improvement of nutrient status. The proportions of aggregate fractions at the sizes of > 2 mm, 0.5~2 mm, and 0.25~0.5 mm increased by 5.1, 18, and 9.2 % of the total soil mass from 2013 to 2015, respectively. The effective formation of macro-aggregates over reclamation time revealed a facilitating effect of the integrated sludge addition and AMF inoculation on pedogenesis process of the mine soil. Further study found that SOC content was closely related to the aggregate fraction of > 2 mm and 0.25~0.5 mm with $r = -0.911$ and -0.90, respectively, implying an important role of aggregation process in the pedogenesis of reconstructed mine soils. The enzyme activities (i.e., invertase, urease, and acid phosphatase activities) were not correlated with the distribution of aggregates, but they were correlated with SOC in the aggregate fraction of 0.25~0.5 mm. The outcomes from the present study are expected to provide an effective option for the reclamation of the mined soil and the stability of aggregates. Further studies are required to improve mine soil reconstruction using the measures including selection of plants with deep root, amendments addition, and mulching.

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Compliance with the ethical standard

Conflict of interest We have no conflict of interest.

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