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Slow Recovery of Major Soil Nutrient Pools during Reclamation in a Sub-Alpine Copper Mine Area, Southeastern Edge of the Tibetan Plateau, Sichuan Province, SW China

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Abstract: Improvement of soil nutrients is crucial for the long-term development and stability of restored ecosystems in mine areas. However, knowledge about the variation in soil nutrients and their influencing factors during the reclamation of sub-alpine metal mine soil is still scarce. We assess the status of soil organic carbon (SOC), total nitrogen (TN), N fractions, total phosphorus (TP), and available P in reclaimed soil at a copper mine site (2702 m above sea level) in the southeastern edge of the Tibetan Plateau, southwest China. The mine area had been reclaimed by distributing stockpiled topsoil (~15 cm) in 2008, sowing seeds of ryegrass (*Lolium perenne* L.) in 2009, and planting seedlings of *A. ferdinandi-coburgii* and *Rosa omeiensis* f. *pteracantha* in 2010. We found that, eight years after the reclamation, although the concentrations of SOC (24.3 g kg⁻¹) and TN (2.21 g kg⁻¹) in the reclaimed soil increased by 25% and 29% compared with the stockpiled topsoil, respectively, they only accounted for about half of the levels in the undisturbed topsoil. In contrast, the concentration of TP (498–570 mg kg⁻¹) did not significantly change between the reclaimed and stockpiled topsoil. The concentrations of NH₄-N (30.1 g kg⁻¹), NO₃-N (17.2 g kg⁻¹), and available P (11.1 mg kg⁻¹) in the reclaimed soil were 2.2, 1.3, and 1.6 times the levels in stockpiled topsoil, respectively, but still lower than those in undisturbed soil. The concentrations of microbial C, N, and P in the reclaimed soil had a similar variation pattern to the available nutrients. The soil C:P and N:P ratios and microbial biomass C:P and N:P ratios in the reclaimed soil were significantly lower than the levels in the undisturbed forest soil. The average accumulation rates of SOC and TN in the reclaimed soil were 85.3 and 11.4 g m⁻² year⁻¹, respectively. The rates are much lower than those of reclaimed mine soils with similar reclaim duration but better climate conditions. Relatively slow development of vegetation and soil microorganisms and leaching due to a freeze–thaw cycle controlled by the sub-alpine climate are likely responsible for the slow recovery of soil SOC and TN.

Keywords: soil organic carbon; total nitrogen; total phosphorus; available nutrients; ecological stoichiometry; reclamation; metal mine tailing

1. Introduction

Mining exploitation has caused irreversible damage to many natural ecosystems around the world. Reclamation is an effective technology to recover a disturbed ecosystem and restore its biodiversity and function [1,2]. Mine soil quality is a key indicator to assess the success of reclamation in mine sites [3–5] because the long-term development and stability of recovered plant communities are based on the systematic development and improvement of soil properties, including microbial functions [6,7]. Organic carbon (C) and nitrogen (N) pools in mine soil are massively degraded

by mining activities [8,9]. Soil nutrients have been identified as the most critical properties when evaluating mine soil quality [5,10–12]. As a consequence, information on the variation in soil nutrients during reclamation is crucial to achieve successful reclamation [13].

A large number of studies have focused on the dynamics of soil physical, chemical, and biological properties during reclamation in several climate types [8,14–21]. These studies have estimated the accumulation rates of soil organic C (SOC), total N (TN), and available C, N, and phosphorus (P), analyzed the major driving factors affecting variation in the nutrients, and discussed the relationships between soil development and revegetation. However, most of the reclaimed mine areas in these studies are located in temperate [8,16,17,19], sub-tropical, and tropical zones [14,15,18,20,21]. Hardly any research has focused on the recovery of major soil nutrients during reclamation in sub-alpine areas.

Sub-alpine regions are prevalently characterized by relatively low temperatures, short growing season, and intensive freeze–thaw cycles [22]. Knowledge about how these features influence the recovery of major soil nutrient pools and the driving factors during reclamation is still scarce. For example, the low temperatures and short growing season presumably do not facilitate the rapid growth of plants, especially woody plants, and thus reduce the return of C by litter. However, because the microbial mineralization of soil organic matter is also inhibited, the low temperature is likely beneficial to SOC sequestration. In addition, freeze–thaw cycles decrease soluble nutrient pools by causing leaching of nutrients from topsoil. In contrast, these factors increase available mineral nutrients since the freeze–thaw cycle effectively breaks the minerals into fine particles, enhancing the weathering of mine soil and, thus, the release of nutrients.

Compared with coal mines, metal mine areas, especially copper mine sites, have more constraints on plant establishment and colonization, such as heavy metal toxicity, low content of nutrients, and extreme pH [23–25]. This highlights the essential role of improvement of soil physical–chemical–biological properties in successful reclamation. In addition, this also is a major cause for the rare field evidence about the variations in soil nutrient pools in copper mine sites in sub-alpine zones [26,27]. In the present study, we investigate the variation in the pools of SOC, N, P, and microbial biomass C, N, and P in an experimental reclamation project of a sub-alpine copper mine site in the southern edge of the Tibetan Plateau. In addition, we estimate the accumulation rates of SOC and N and analyze the changes in C:N:P stoichiometry. The first goal is to assess the status of SOC, N, and P in the reclaimed mine soil after eight years' reclamation at the sub-alpine copper mine site. The second goal is to elucidate the effects of revegetation and sub-alpine climate on the available nutrient pools and the accumulation rates of SOC and TN in the reclamation soil.

2. Materials and Methods

2.1. Site Description

This study was conducted at an experimental reclamation project in a copper mine (chalcopyrite) (28°26'49" E, 101°41'43" E, 2702 m above sea level) in the southeastern edge of the Tibetan Plateau, southwest China (Figure S1). The protolith of this mine is a set of argillaceous-arenaceous marine sedimentary rocks in the passive continental margin, containing a small amount of volcanic rocks. Metallogenic materials mainly come from the ore-bearing rocks characterized by multiple phases and many geneses. The formation occurred during the Yanshan period (133–143 Ma) by middle to high hydrothermal fluid mineralization [28]. The mean annual temperature is 7 °C (Table 1). The diurnal temperature range is 21 °C with the maximum annual temperature of 21 °C in July and the minimum annual temperature of −15.6 °C in January (Table 1). The mean annual precipitation of 802 mm is concentrated in the summer season (Table 1). Soil freeze–thaw cycles occur from November to March (Table 1), showing a cryic temperature regime to pedogenic processes in this region. This climate regime is typical and common in the sub-alpine region in the southeastern edge of the Tibetan Plateau. The undisturbed forest in the vicinity of the mining area is dominated by alder (*Alnus ferdinandi-coburgii*) with a height of 2–10 m. The coverage percent of the undisturbed forests is 90–100% (Table 1). The soil

in the undisturbed forest is classified as leached brown soil according to the Genetic Soil Classification of China (GSCC) [29] or Alfisols according to soil taxonomy (ST) [30]. The slope of the reclamation area is 5–10° facing southwest. The experimental reclamation action was to cover stockpiled topsoil ~15 cm deep in 2008. The topsoil was taken from the surface mineral soil (0–30 cm) before this area was mined. In April 2009, seeds of ryegrass (*Lolium perenne* L.) were sown. In April 2010, seedlings of *A. ferdinandi-coburgii* and *Rosa omeiensis* f. *pteracantha* were planted with a distance of about 5 m to each other. The survival rate of the two species was recorded as between 30% and 40% in 2017. The coverage percent of the restoration vegetation was 30–50% (Table 1). This reclamation method is common in most mine areas in the southeastern edge of the Tibetan Plateau.

Table 1. Characteristics of soil sampled at two depths and vegetation at different sites in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau.

Sites/Samples	Depth (cm)	Description	BD (g cm ⁻³)	Sand (%)	Silt	Clay	pH	Vegetation/Climate	Coverage (%)
Reclaimed site	0–15 15–30	R _s , reclaimed soil R _{sp} , reclaimed spoil	1.20 1.87	56.0 a 70.7 b	42.0 a 27.9 b	2.0 a 1.4 b	5.7 a 4.9 b	<i>Alnus ferdinandi-coburgii</i> (height: 1–2.5 m); <i>Rosa omeiensis</i> f. <i>pteracantha</i> (0.4–0.8 m); <i>Lolium perenne</i> L.	30–50
Undisturbed forest	0–15 15–30	U _{top} , undisturbed top soil U _{sub} , undisturbed subsurface soil	1.14 1.23	49.7 c 40.9 d	47.3 c 55.8 d	3.0 c 3.3 c	5.2 b,c 5.3 c	<i>Alnus ferdinandi-coburgii</i> (height: 2–10 m)	90–100
Initial soil		CK _s , top soil used to reclamation	1.32	55.2 a	42.3 a	2.5 d	5.9 a	MAT: 7 °C, MDTR: 16 °C, MaxAT: 21 °C (Jul.)	
Initial mine spoil		CK _{sp}	1.95	74.2 b	25.0 b	0.8 e	3.8 d	MinAT: −15.6 °C (Jan.) MAP: 802 mm Freeze-thaw: Nov.–Mar.	

BD: bulk density. MAT: mean annual temperature, MDTR: mean diurnal temperature range, MaxAT: maximum annual temperature, MinAT: minimum annual temperature, MAP: mean annual precipitation. Data of soil particle size are shown as means of 6 replicates. Different letters indicate significantly different contents of sand, silt, and clay between different sites at the $p < 0.05$ level.

2.2. Sampling and Analyses

In the reclaimed area, six plots (5 × 5 m) with a distance of >30 m from each other were selected to collect soil samples. In each plot, ten soil cores were collected by a soil auger. The depth of each soil core was 30 cm. The cores were divided into two layers to collect samples: 0–15 cm—reclaimed soil (R_s) and 15–30 cm—reclaimed spoil (R_{sp}) (Figure S2). The collected sub-samples were mixed thoroughly to a composite sample for each horizon at each plot. Thus, we collected two composite samples from each plot, i.e., data of 12 composite samples from the reclaimed area. A litter horizon was not found in the reclaimed plots, therefore we did not collect litter samples.

In addition to R_s and R_{sp}, soil samples were also collected from the adjacent undisturbed forests (U_{top}: 0–15 cm, U_{sub}: 15–30 cm) as reference soil (Figure S2). The initial stockpiled topsoil (CK_s) and mine spoil (CK_{sp}) were collected as control groups (Figure S2). Soil samples were stored in a cooler with ice (1–4 °C) before moving to a laboratory. Samples for the analysis of soil properties were sieved (<2 mm) to remove coarse rocks and roots. Then, the soil samples were divided into two parts and were air-dried or stored at 4 °C (fresh soils), respectively.

Soil bulk density (BD) was determined by excavating holes with a certain volume. The volume of excavated soil could be measured by backfilling with a known volume of water [31]. Samples for BD were oven-dried at 105 °C for 48 h, and the weight and volume of coarse fragment (>2 mm) were subtracted from the total soil weight and volume to calculate BD. Air-dried soil samples were used for the rest of analyses except for determining NH₄-N, NO₃-N and microbial biomass C, N, and P (MBC, MBN, and MBP). Soil pH was determined in H₂O suspensions (soil:water ratio of 1:2.5) using a pH meter (FE20K, Mettler Toledo, Germany). The particle size distribution was determined by a laser particle size analyzer (Mastersizer 2000) after samples were treated with 3% H₂O₂ (clay: <2 µm; silt: 2–20 µm; sand: 20–2000 µm). SOC and total N (TN) concentrations were measured using an

element analyzer (Elementar vario MACRO cube, Germany). Total P (TP) was measured by a profile DV (USA Teledyne Leeman Labs) inductively coupled plasma atomic emission spectrometer (ICP-AES) (detection limit: 0.02 mg P/L) after digestion by refluxing with the nitric acid (6 mL), hydrofluoric acid (2 mL), and perchloric acid (1 mL). Available P was determined using the method proposed by Olsen and Sommers (1982) [32]. The concentrations of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were determined using a flow autoanalyzer (SEAL analytical AutoAnalyzer 3, Germany) after extraction by 1 M KCl solution. The contents of MBC, MBN, and MBP were measured by the chloroform fumigation-extraction method [33,34]. Two replicates were used when determining the above soil properties.

2.3. Calculations

The pools of SOC and TN (Pool, kg m^{-2}) in different soil horizons were calculated using the following formula:

$$\text{Pool} = (\text{Conc.} \times \text{BD} \times D)/100 \quad (1)$$

where Conc., BD and D are the concentration of SOC or TN (g kg^{-1}), bulk density (g m^{-3}), and depth (cm) in each horizon, respectively.

The accumulation rate of SOC and TN (R , $\text{g m}^{-2} \text{year}^{-1}$) was calculated using the following formula:

$$R = \text{Pool}/\text{Age} \times 10 \quad (2)$$

where Age is the reclamation time (8 years).

2.4. Statistics

A test of homogeneity of variances was applied to pH, the contents of sand, silt, and clay and the concentrations of SOC, TN, TP, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, MBC, MBN, and MBP (Table S1). If the variance of a variable was not homogeneous, the variable was transformed by the natural logarithm function. Variables were subjected to a one-way analysis of variance (ANOVA) to test differences between R_S , R_{SP} , CK_S , CK_{SP} , U_{TOP} , and U_{SUB} (Table S2), after which LSD's post-hoc tests were performed (Table S3). Before a correlation analysis was applied, variables were tested for normality (Q-Q plots, Figure S3). The correlations between these variables were determined by Pearson's correlation coefficients. A significance level of $p < 0.05$ was used in this study (except where noted).

3. Results

3.1. Soil Properties

The bulk density in the reclaimed soil (R_S) was lower than that in the initial stockpiled topsoil (CK_S) but higher than that in the undisturbed topsoil (U_{TOP}) (Table 1). The proportion of clay in the R_S was lower than that in the CK_S and U_{TOP} . The reclaimed spoil (R_{SP}) had more clay than the initial mine spoil (CK_{SP}). We found no significant difference of pH between the R_S and CK_S . In contrast, pH of the R_{SP} increased by 1.1 units compared with the CK_{SP} (Table 1).

3.2. Variations in soil C, N and P

The concentrations of SOC ($24.3 \pm 1.7 \text{ g kg}^{-1}$, mean $\pm$ SD) and TN ($2.21 \pm 0.12 \text{ g kg}^{-1}$) in the R_s increased significantly compared with the CK_s (SOC: $19.4 \pm 1.6 \text{ g kg}^{-1}$, TN: $1.71 \pm 0.19 \text{ g kg}^{-1}$), but only accounted for 44% and 54% of those in the U_{top} , respectively (Figure 1a,b). The concentrations of SOC ($2.46 \pm 0.21 \text{ g kg}^{-1}$) and TN ($0.57 \pm 0.03 \text{ g kg}^{-1}$) in the R_{sp} increased slightly compared with the CK_{sp} (SOC: $1.81 \pm 0.24 \text{ g kg}^{-1}$, TN: $0.43 \pm 0.06 \text{ g kg}^{-1}$). The concentrations of $NH_4\text{-N}$ ($30.1 \pm 5.8 \text{ g kg}^{-1}$) and $NO_3\text{-N}$ ($17.2 \pm 3.7 \text{ g kg}^{-1}$) in the R_s were 2.2 and 1.3 times of those in the CK_s (Figure 2a,b). The concentrations of $NH_4\text{-N}$ and $NO_3\text{-N}$ increased from 3.2 and 0.8 mg kg^{-1} in the CK_{sp} to 13.2 and 5.5 mg kg^{-1} in the R_{sp} , respectively. The concentration of $NH_4\text{-N}$ was higher than that of $NO_3\text{-N}$ in both the R_s and R_{sp} . However, there was higher concentration of $NO_3\text{-N}$ than $NH_4\text{-N}$ in both the U_{top} and undisturbed subsurface soil (U_{sub}) (Figure 2a,b).

Figure 1. Comparisons of the concentrations of (a) soil organic carbon (SOC), (b) total nitrogen (TN), and (c) total phosphorus (TP) between the reclaimed soil (R_s), reclaimed spoil (R_{sp}), initial topsoil (CK_s), initial spoil (CK_{sp}), and adjacent undisturbed forest soils (0–15 cm, U_{top} ; 15–30 cm, U_{sub}) in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau. Data are shown as means $\pm$ SD of 6 replicates. Different letters indicate significantly different variables between different samples at the $p < 0.05$ level.

Figure 2. Comparisons of the concentrations of (a) $NH_4\text{-N}$, (b) $NO_3\text{-N}$, and (c) available P between the reclaimed soil (R_s), reclaimed spoil (R_{sp}), initial topsoil (CK_s), initial spoil (CK_{sp}), and adjacent undisturbed forest soils (0–15 cm, U_{top} ; 15–30 cm, U_{sub}) in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau. Data are shown as means $\pm$ SD of 6 replicates. Different letters indicate significantly different variables between different samples at the $p < 0.05$ level.

The lowest concentration of TP ($498 \pm 42 \text{ mg kg}^{-1}$) occurred in the CK_{SP} and the highest one ($570 \pm 46 \text{ mg kg}^{-1}$) occurred in the U_{SUB} (Figure 1c). The concentrations of TP in the R_S ($547 \pm 22 \text{ mg kg}^{-1}$) and R_{SP} ($499 \pm 43 \text{ mg kg}^{-1}$) did not significantly change compared with the CK_S ($565 \pm 34 \text{ mg kg}^{-1}$) and CK_{SP} ($498 \pm 42 \text{ mg kg}^{-1}$). However, the concentrations of available P in the R_S ($11.1 \pm 2.2 \text{ mg kg}^{-1}$) were significantly higher than those in the CK_S ($7.2 \pm 1.4 \text{ mg kg}^{-1}$) and were comparable with the U_{TOP} ($14.4 \pm 4.5 \text{ mg kg}^{-1}$) (Figure 2c).

The C:N ratios (mass ratio) in the R_S (11.0 ± 1.1) and R_{SP} (4.3 ± 0.3) did not significantly change compared with the CK_S (11.4 ± 0.8) and CK_{SP} (4.2 ± 0.3). They also were lower than the U_{TOP} (13.4 ± 0.6) and U_{SUB} (14.4 ± 0.4) (Figure 3a). In contrast, the C:P (44.3 ± 3.7) and N:P (4.1 ± 0.4) ratios in the R_S were significantly higher than those in the CK_S (34.4 ± 1.5 and 3.0 ± 0.3), although they were still largely lower than those in the U_{TOP} (105.8 ± 10.7 and 7.9 ± 0.8) (Figure 3b,c).

Figure 3. Comparisons of (a) C:N, (b) C:P, and (c) N:P ratios (mass ratio) between the reclaimed soil (R_s), reclaimed spoil (R_{sp}), initial topsoil (CK_s), initial spoil (CK_{sp}), and adjacent undisturbed forest soils (0–15 cm, U_{top}; 15–30 cm, U_{sub}) in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau. The blue dash line represents the value of global soil C:N, C:P, and N:P ratios [35]. The orange dash line represents the value of soil C:N, C:P, and N:P ratios in China [36]. Data are shown as means $\pm$ SD of 6 replicates. Different letters indicate significantly different variables between different samples at the $p < 0.05$ level.

3.3. Variations in Soil Microbial Biomass C, N, and P

MBC, MBN, and MBP showed a similar pattern between different groups (Figure 4a–c). The concentrations of MBC, MBN, and MBP in the R_S increased largely compared the CK_S. Although the concentrations of MBC ($220 \pm 22 \text{ mg kg}^{-1}$), MBN ($22.9 \pm 3.0 \text{ mg kg}^{-1}$), and MBP ($8.7 \pm 1.3 \text{ mg kg}^{-1}$) in the R_S were still lower than those in the U_{TOP} (602 ± 59 , 83.1 ± 9.3 and $18.3 \pm 3.4 \text{ mg kg}^{-1}$), they were comparable to those in the U_{SUB} (210 ± 35 , 22.4 ± 4.9 and $5.5 \pm 0.9 \text{ mg kg}^{-1}$). MBC:N (9.8 ± 1.4), MBC:P (25.9 ± 4.2), and MBN:P (2.6 ± 0.1) ratios in the R_S were higher than those in the CK_S and R_{SP} (Figure 5a–c). Compared with the U_{TOP}, the R_S had a comparable MBC:N ratio but lower MBC:P and MBN:P ratio. The concentration of MBC, MBN, and MBP in the CK_{SP} was not detected.

Figure 4. Comparisons of the concentrations of (a) microbial biomass carbon (MBC), (b) microbial biomass nitrogen (MBN), and (c) microbial biomass phosphorus (MBP) between the reclaimed soil (R_s), reclaimed spoil (R_{sp}), initial topsoil (CK_s), and adjacent undisturbed forest soils (0–15 cm, U_{top}; 15–30 cm, U_{sub}) in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau. The concentration of MBC, MBN, and MBP in the initial spoil (CK_{sp}) was not detected. Data are shown as means $\pm$ SD of 6 replicates. Different letters indicate significantly different variables between different samples at the $p < 0.05$ level.

Figure 5. Comparisons of (a) microbial biomass carbon to nitrogen (MBC:N), (b) microbial biomass carbon to phosphorus (MBC:P), and (c) microbial biomass nitrogen to phosphorus (MBN:P) ratios (mass ratio) between the reclaimed soil (R_s), reclaimed spoil (R_{sp}), initial topsoil (CK_s), and adjacent undisturbed forest soils (0–15 cm, U_{top}; 15–30 cm, U_{sub}) in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau. The blue dash line represents the value of global soil MBC:N, MBC:P, and MBN:P ratios [35]. Data are shown as means $\pm$ SD of 6 replicates. Different letters indicate significantly different variables between different samples at the $p < 0.05$ level.

3.4. Rate of Accumulation of Soil C and N

After eight years of reclamation, the SOC and TN pools increased from 3.85 and 0.34 kg m⁻² in the CK_s to 4.37 and 0.40 kg m⁻² in the R_s, respectively (Figure 6). The SOC and TN pools in the R_{sp} increased by 0.16 and 0.03 kg m⁻² compared with those in the CK_{sp}, respectively. The SOC and TN pools in the R_s were 46% and 56% of those in the U_{top}. For the R_s, the accumulation rates of SOC and TN were 65.0 and 7.3 g m⁻² year⁻¹, respectively (Table 2). For the R_{sp}, the rates were 20.3 (SOC) and 4.1 (TN) g m⁻² year⁻¹, respectively.

Figure 6. Comparisons of the pools of soil organic carbon (SOC) and total nitrogen (TN) between the reclaimed soil (0–15 cm, R_s), reclaimed spoil (15–30 cm, R_{sp}), initial topsoil (CK_s), initial spoil (CK_{sp}), and adjacent undisturbed forest soils (0–15 cm, U_{top}; 15–30 cm, U_{sub}) in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau.

4. Discussion

4.1. Variation in Soil Properties

The decrease in the bulk density of the R_s compared with the CK_s is likely a result of the accumulation of SOC with revegetation (Table 1, Figure 1). The decline of bulk density caused by the input of organic matter occurs in many young natural and recovered ecosystems. For example, soil bulk density decreased with increasing SOC along natural chronosequences in the Tibetan Plateau [37] and in the European Alps [38]. The decline of bulk density with the restoration period has also been reported in many recovered ecosystems [8,14,39,40]. The increase in pH of the R_{sp} compared with the CK_{sp} (Table 1) is probably caused by the deposition of base cations leached from the upper R_s.

4.2. Variation in Major soil nutrients

Comparing the R_s with CK_s, the significant accumulation of both SOC and TN (Figure 1a,b, and Figure 6) and the slight decrease in TP (Figure 1c) showed a decoupled variation with the process of reclamation. The insignificant difference between the C:N ratios (Figure 3a) and the distinct changes in the C:P and N:P ratios (Figure 3b,c) between the R_s and CK_s also indicate this decoupled variation. In addition, the strong correlations between SOC, TN, MBC, and MBN and no correlation between TP and SOC, TN, MBC, MBN, and MBP (Table S3) further show the divergent variation in SOC and TN and TP.

This difference is presumably because of the different ultimate sources and biogeochemical cycling of C, N, and P. Indeed, C, N, and P all can be returned to the soil by the litter of *Lolium perenne* L. and *Astragalus membranaceus* in our reclaimed site. However, the ultimate major sources of organic C and N in the soil are atmospheric through photosynthesis and fixation by plants and microorganisms, while the ultimate source of P is the weathering of primary mineral P [41,42]. As a result, these differences will gradually increase the C and N pools in soil but only “cycle” P from bottom to top horizons. More than that, the P content in soil tends to decrease with long-term pedogenesis [43]. Consequently, similar to our results, increasing C and N and decreasing soil P with the pedogenic process have been observed in many natural and reclaimed ecosystems in various climate types [7,14–16,37,44,45].

Table 2. Pools and accumulation rates of SOC and TN in reclaimed soils in mine areas with similar reclamation duration in the world.

No.	Site	Duration (years)	R _{SOC} (g m ⁻² .year ⁻¹)	R _{TN} (g m ⁻² .year ⁻¹)	MAT (°C)	MAP (mm)	Soil Depth (cm)	Vegetation	Mine Type	Source
1	Sichuan, SW China	8	65.0	7.3	7	802	0–15	<i>Alnus ferdinandi-coburgii</i> (height: 1–2.5 m); <i>Rosa omeiensis</i> f. <i>pteracantha</i> (0.4–0.8 m); <i>Lolium perenne</i> L.	Copper	This study
			20.3	4.1			15–30			
	Total		85.3	11.4			0–30			
	Western North Dakota	45	13.1	2.5	6	453	0–70	<i>Bromus inermis</i> , <i>Melilotus</i> spp.	Lignite	[46]
	Singrauli, India	5	16.0	1.5	23	1069	0–20	<i>Tectona grandis</i> L.f	Coal	[18]
2A			249	4.9				<i>Albizia lebbek</i> (L.) Benth.		
2B			229	11.2				<i>A. procera</i> (Roxb.) Benth.		
2C			324	20.0				<i>Dendrocalamus strictus</i>		
2D			78.2	29.7				<i>Pinus sylvestris</i> L, <i>Betula pendula</i> L.		
3	Southern Poland	5	102		8	700	0–20	Pasture Pasture Mixed hardwood species	Sand	[47]
4A	SE Ohio, USA	5	239		11	1020	0–30		Coal	[8]
4B		10	90				0–30		Lignite	
4C		10	270				0–30			
5A		12	520				0–110			
5B	Central Poland	17	171	2.0	8	580	0–110			[17]
6	Jharkhand, India	4th to 7th	260		26	1375	0–30	Shrub and forest	Coal	[14]
7	Telangana, India	8			22–33	975	0–60	Forest with herbs	Coal	[15]
8	West Virginia, USA	5th to 7th		22.2	19	1120	0–15	<i>Robinia pseudoacacia</i> L.	Coal	[48]

Duration: duration of reclamation; R_{SOC}: accumulation rate of SOC; R_{TN}: accumulation rate of TN; MAT: mean annual temperature; MAP: maximum annual precipitation.

The increase in the concentrations of $\text{NH}_4\text{-N}$ and available P in the R_S (Figure 2a–c) reflect the improvement of soil available nutrient pools after revegetation. This is likely related to the increase in soil microbial activity according to their strong correlations to MBC, MBN, and MBP (Table S4). The ratio of MBC to SOC (MBC/SOC) increased from 0.63% in the CK_S to 0.91% in the R_S demonstrates the enhanced microbial activity due to revegetation. The high ratios of MBN to $\text{NH}_4\text{-N}$ (0.76) and $\text{NO}_3\text{-N}$ (0.51) also show the important role of microorganisms in transforming N [49]. In addition, the positive correlations of available P to SOC, TN, MBC, MBN, and MBP but not to TP (Table S4) and the high ratio of MBP to available P (0.78) further indicate that microbial transformation is a major mechanism of the increase in available P. Previous studies also related variations in soil nutrient status to microbial activities [19].

4.3. Accumulation Rates of C and N

The accumulation rate of SOC at our site is higher than that at a lignite mine site in Western North Dakota (No. 1 in Table 2, [46]) and a coal mine site in India planted with *Tectona grandis* L.f (No. 2A in Table 2, [18]), slightly higher than that at a sand mine site in Southern Poland (No. 3 in Table 2, [47]), comparable to that at a coal mine site recovered by forest in Southeastern Ohio, USA (No. 4C in Table 2, 10 years, [8]), and lower than that at other sites with a similar reclamation duration but higher MAT and MAP (No. 2B–D, 4A, 4B, 5A, 5B, 6, and 7 in Table 2, [8,14,15,17,18]). Our accumulation rate of TN is in the middle of the range ($1.5\text{--}29.7\text{ g m}^{-2}\cdot\text{year}^{-1}$) of the previous studies with various climate and vegetation types (Table 2).

The differences of the rates of SOC and TN accumulation between our site and other sites can be jointly attributed to the effects of climate and vegetation type. First, relatively higher precipitation and temperature favor the development of vegetation and microbial activities, which further facilitate the fixation of N and the return of C and N to soil. This can be reflected by the observation that higher accumulation rates of SOC and TN generally occurred in sites with higher MAT and MAP (Table 2). Second, vegetation type and its interaction with climate can also influence the accumulation of SOC and TN. This can be shown by that the accumulation rates of SOC and TN in our site are even higher than those in several sites with higher MAT and MAP (No. 2A, 3, and 6 in Table 2). The large differences (11-fold) of the accumulation rate of TN between the Sites 6 and 8 further show the influence of vegetation type. The MAT and MAP are comparable at the two sites but Site 8 was dominated by the locust (leguminous tree) while Site 6 was dominated by non-leguminous species (Table 2). Singh et al. (2004) also found that leguminous trees cause a larger increase in soil N pool than non-leguminous trees [50]. In addition, the relatively high content of total Cu in the R_{SP} ($\sim 368\text{ mg/kg}$, personal communication) maybe prevent the growth of plants and activity of microbes, inhibiting the accumulation of SOC and TN.

The accumulation rates of SOC and TN at our site were largely higher than those in natural soil during primary succession, similar to some previous reports [20,47]. For example, our accumulation rate of SOC is 2.6–12.0 times of that at three “young” chronosequences (120–150 years) in the Swiss Alps [51–53]. Our accumulation rate of TN is 2.7–5.7 times of that at a lava chronosequence (142 years) in tropical Hawaii [44]. This is no surprise because the parent materials of natural successional soils are often lacking in C and N [52], while the reclaimed soil often has a certain amount of legacy C and N (Figure 1a,b), which can facilitate the rapid establishment of microorganisms and vegetation. As a consequence, considerable amounts of SOC and N can be rapidly fixed or returned to soil by litters in reclaimed sites.

4.4. Slow Increase in Soil Nutrient Pools in Sub-Alpine Mine Site

For our reclaimed soil, it would take around 124 years to reach SOC equivalence with the undisturbed forest soil, if keeping the current rate (Table 2). Because many previous studies have reported that the accumulation rate of SOC decreases with soil development [45,51], the actual recovery duration of SOC would be longer than this estimation. This recovery duration is much longer than

that found in reclaimed sites with better climate conditions [4,14,40]. For example, the reclaimed site would need less than 20 years to achieve the SOC content of the reference forest in tropical Ranchi district, India [14]. Mukhopadhyay et al. (2014) found that soil quality after 17 years reclamation was comparable to that in a nearby undisturbed forest in a tropical coal mine site in India [4]. For a coal mine in a temperate zone, northern China, Yuan et al. (2018) found that the duration required to reach the nutrient level of undisturbed site was about 10 years [40].

The slow increase in soil SOC and TN at our site is likely related to the relatively slow development of vegetation (Table 1) and microbial activities controlled by the relatively cold and dry climate. This point can be supported by the variation in available nutrients, microbial biomass, and C:N:P ratios. First, although the concentrations of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ at the R_S increased compared with the CK_S , they only accounted for 67.5% and 26.6% of those in the U_top . The lower content of $\text{NO}_3\text{-N}$ content than $\text{NH}_4\text{-N}$ in the R_S further illustrates that it will need a long time to reach the level of nutrients at the undisturbed site where the content of $\text{NO}_3\text{-N}$ is higher than $\text{NH}_4\text{-N}$ (Figure 2a,b). The low temperature and the long frost period (Table 1) is probably an important factor causing weak nitrification and, thus, the low content of $\text{NO}_3\text{-N}$ in the R_S . In addition, the leaching of soluble $\text{NO}_3\text{-N}$ is also likely responsible for the low content of $\text{NO}_3\text{-N}$ in R_S . This can be shown by the existence of $\text{NO}_3\text{-N}$ in the subsurface R_SP (Figure 2b). The undetectable MBC and MBN in the R_SP indicate that the $\text{NO}_3\text{-N}$ in the R_SP came from the upper R_S horizon by leaching. The occurrence of leaching at our reclaimed site is also demonstrated by the increase in clay content of the R_SP and the decrease in clay content of the R_S (Table 1).

Second, the concentrations of MBC, MBN, and MBP in the R_S were 36.5%, 27.5%, and 47.3% of those in the U_TOP (Figure 4a–c), showing the relatively low rate of microbial nutrient transformation controlled by the low temperature. This is also reflected by the observation that the MBC/SOC (0.9%), MBN/TN (1%), and MBP/TP (1.6%) in the R_S were lower than those in the U_TOP , and much lower than those in various types of soils around the world [54–57]. In addition, even in low altitudinal areas, it will take several decades or more for microbial biomass and diversity in disturbed soils to reach the levels of undisturbed soils [58,59]. Therefore, the slow recovery of soil microbial function is likely a reason for the slow recovery of soil nutrient pools at our site.

Finally, although the C:N ratio in the R_S approached the ratios in China and global soils (Figure 3a) [35,36], the much lower C:P and N:P ratios than the global values (Figure 3b,c) indicate the insufficient contents of SOC and N in the R_S . Similar to the soil C:N:P ratios, the MBC:N ratio in the R_S increased to the same level of the U_TOP (Figure 5a); however, the MBC:P ratio is slightly lower than the global value and the MBN:P ratio much lower than that in the U_TOP , U_SUB , and global level (Figure 5a) [35]. These results illustrate not only the lack of C and N in the R_S , but also the likely N limitation to the microbial community.

5. Conclusions

The SOC and TN pools in the reclaimed soil after eight years of reclamation increased significantly, compared with the initial stockpiled topsoil in the sub-alpine copper mine area, southeastern edge of the Tibetan Plateau, southwest China. In contrast, a significant difference in the TP pool was not observed. The SOC and TN pools in the reclaimed soil accounted for 46% and 56% of those in the adjacent undisturbed forest soil. The concentrations of MBC, MBN, and MBP in the reclaimed soil were only 37%, 27%, and 48% of the levels in the undisturbed soil, respectively. The relatively slow increase in soil SOC and TN pools are likely related to the relatively cold and dry sub-alpine climate, which does not favor the fast development of vegetation and microbial activities.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/10/12/1069/s1>, Figure S1: The location of the reclaimed area and the undisturbed forest in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau, Figure S2: Six classes of soil and spoil samples in this study, Figure S3: Q-Q plots of 11 variables in a sub-alpine copper mine site, southeastern edge of the Tibetan Plateau, Table S1: Test of Homogeneity of Variances, Table S2: Results of ANOVA analysis of 11 variables, Table S3: Pearson's correlation coefficients between the concentrations of soil C, N, P, microbial biomass C, N and P (MBC, MBN and MBP) in a sub-alpine copper mine site, southern edge of the Tibetan Plateau ($N = 30$), Table S4: Pearson's correlation coefficients between the concentrations of available P, $\text{NH}_4\text{-N}$ and $\text{NH}_4\text{-N}$ and the soil C, N, P, microbial biomass C, N and P (MBC, MBN and MBP) in a sub-alpine copper mine site, southern edge of the Tibetan Plateau ($N = 30$).

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References

- Li, M.S. Ecological restoration of mineland with particular reference to the metalliferous mine wasteland in China: A review of research and practice. *Sci. Total Environ.* **2006**, *357*, 38–53. [CrossRef]
- Wang, L.; Ji, B.; Hu, Y.H.; Liu, R.Q.; Sun, W. A review on in situ phytoremediation of mine tailings. *Chemosphere* **2017**, *184*, 594–600. [CrossRef]
- Yao, R.J.; Yang, J.S.; Gao, P.; Zhang, J.B.; Jin, W.H. Determining minimum data set for soil quality assessment of typical salt-affected farmland in the coastal reclamation area. *Soil Tillage Res.* **2013**, *128*, 137–148. [CrossRef]
- Mukhopadhyay, S.; Maiti, S.K.; Masto, R.E. Development of mine soil quality index (MSQI) for evaluation of reclamation success: A chronosequence study. *Ecol. Eng.* **2014**, *71*, 10–20. [CrossRef]
- Mukhopadhyay, S.; Masto, R.E.; Yadav, A.; George, J.; Ram, L.C.; Shukla, S.P. Soil quality index for evaluation of reclaimed coal mine spoil. *Sci. Total Environ.* **2016**, *542*, 540–550. [CrossRef] [PubMed]
- Li, X.F.; Huang, L.B. Toward a New Paradigm for tailings phytostabilization-nature of the substrates, amendment options, and anthropogenic pedogenesis. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45*, 813–839. [CrossRef]
- Shrestha, R.K.; Lal, R. Changes in physical and chemical properties of soil after surface mining and reclamation. *Geoderma* **2011**, *161*, 168–176. [CrossRef]
- Akala, V.A.; Lal, R. Soil organic carbon pools and sequestration rates in reclaimed minesoils in Ohio. *J. Environ. Qual.* **2001**, *30*, 2098–2104. [CrossRef] [PubMed]
- Ganjugunte, G.K.; Wick, A.F.; Stahl, P.D.; Vance, G.F. Accumulation and composition of total organic carbon in reclaimed coal mine lands. *Land Degrad. Dev.* **2009**, *20*, 156–175. [CrossRef]
- Bodlák, L.; Krováková, K.; Kobesová, M.; Brom, J.; Stastný, J.; Pecharová, E. SOC content—An appropriate tool for evaluating the soil quality in a reclaimed post-mining landscape. *Ecol. Eng.* **2012**, *43*, 53–59. [CrossRef]
- Chaudhuri, S.; McDonald, L.M.; Skousen, J.; Pena-Yewtukhiw, E.M. Soil organic carbon molecular properties: Effects of time since reclamation in a minesoil chronosequence. *Land Degrad. Dev.* **2015**, *26*, 237–248. [CrossRef]
- Monserie, M.F.; Watteau, F.; Villemin, G.; Ouvrard, S.; Morel, J.L. Technosol genesis: Identification of organo-mineral associations in a young Technosol derived from coking plant waste materials. *J. Soil Sediment* **2009**, *9*, 537–546. [CrossRef]
- Courtney, R.; Mullen, G.; Harrington, T. An Evaluation of revegetation success on bauxite residue. *Restor. Ecol.* **2009**, *17*, 350–358. [CrossRef]
- Ahirwal, J.; Maiti, S.K.; Singh, A.K. Changes in ecosystem carbon pool and soil CO_2 flux following post-mine reclamation in dry tropical environment, India. *Sci. Total Environ.* **2017**, *583*, 153–162. [CrossRef]

15. Ahirwal, J.; Maiti, S.K.; Reddy, M.S. Development of carbon, nitrogen and phosphate stocks of reclaimed coal mine soil within 8 years after forestation with *Prosopis juliflora* (Sw.) Dc. *Catena* **2017**, *156*, 42–50. [[CrossRef](#)]
16. Liu, X.Y.; Bai, Z.K.; Zhou, W.; Cao, Y.G.; Zhang, G.J. Changes in soil properties in the soil profile after mining and reclamation in an opencast coal mine on the Loess Plateau, China. *Ecol. Eng.* **2017**, *98*, 228–239. [[CrossRef](#)]
17. Pietrzykowski, M.; Daniels, W.L. Estimation of carbon sequestration by pine (*Pinus sylvestris* L.) ecosystems developed on reforested post-mining sites in Poland on differing mine soil substrates. *Ecol. Eng.* **2014**, *73*, 209–218. [[CrossRef](#)]
18. Singh, A.; Zeng, D.; Chen, F. Effect of young woody plantations on carbon and nutrient accretion rates in a redeveloping soil on coalmine spoil in a dry tropical environment, India. *Land Degrad. Dev.* **2006**, *17*, 13–21. [[CrossRef](#)]
19. Šourková, M.; Frouz, J.; Fettweis, U.; Bens, O.; Hüttel, R.; Šantrůčková, H. Soil development and properties of microbial biomass succession in reclaimed post mining sites near Sokolov (Czech Republic) and near Cottbus (Germany). *Geoderma* **2005**, *129*, 73–80. [[CrossRef](#)]
20. You, F.; Dalal, R.; Huang, L.B. Initiation of soil formation in weathered sulfidic Cu-Pb-Zn tailings under subtropical and semi-arid climatic conditions. *Chemosphere* **2018**, *204*, 318–326. [[CrossRef](#)]
21. Yuan, M.; Xu, Z.P.; Baumgartl, T.; Huang, L. Organic amendment and plant growth improved aggregation in Cu/Pb-Zn tailings. *Soil Sci. Soc. Am. J.* **2016**, *80*, 27–37. [[CrossRef](#)]
22. Zhong, X.H.; Zhang, W.J.; Luo, J. The characteristics of the mountain ecosystem and environment in the Gongga Mountain region. *Ambio* **1999**, *28*, 648–654.
23. Das, M.; Maiti, S.K. Comparison between availability of heavy metals in dry and wetland tailing of an abandoned copper tailing pond. *Environ. Monit. Assess.* **2008**, *137*, 343–350. [[CrossRef](#)] [[PubMed](#)]
24. Fernández-Calviño, D.; Cutillas-Barreiro, L.; Nóvoa-Muñoz, J.C.; Díaz-Raviña, M.; Fernández-Sanjurjo, M.J.; Álvarez-Rodríguez, E.; Núñez-Delgado, A.; Arias-Estévez, M.; Rousk, J. Using pine bark and mussel shell amendments to reclaim microbial functions in a Cu polluted acid mine soil. *Appl. Soil Ecol.* **2018**, *127*, 102–111. [[CrossRef](#)]
25. Ye, Z.; Shu, W.; Zhang, Z.; Lan, C.; Wong, M. Evaluation of major constraints to revegetation of lead/zinc mine tailings using bioassay techniques. *Chemosphere* **2002**, *47*, 1103–1111. [[CrossRef](#)]
26. Huang, L.; Baumgartl, T.; Mulligan, D. Is rhizosphere remediation sufficient for sustainable revegetation of mine tailings? *Ann. Bot.* **2012**, *110*, 223–238. [[CrossRef](#)]
27. Mendez, M.O.; Maier, R.M. Phytostabilization of mine tailings in arid and semiarid environments—An emerging remediation technology. *Environ. Health Perspect.* **2007**, *116*, 278–283. [[CrossRef](#)]
28. Li, T.Z.; Feng, X.L.; Zhang, H.H.; Tang, G.L.; Wu, Z.B.; Xia, X.B. Geochemical characteristics of the ore-bearing rock series and genesis of the Liwu copper deposit, Sichuan Province. *Geol. Explor.* **2010**, *46*, 921–930. (In Chinese with English abstract)
29. National Soil Survey Office. *Soils of China*; Chinese Agriculture Press: Beijing, China, 1998; pp. 1–1253. (In Chinese)
30. Soil Survey Staff in USDA. *Keys to Soil Taxonomy*, 12th ed.; USDA—Natural Resources Conservation Service: Washington, DC, USA, 2014; pp. 1–306.
31. Maynard, D.G.; Curran, M.P. Soil density measurement in forest soils. In *Soil Sampling and Methods of Analysis*, 2nd ed.; Carter, M.R., Gregorich, E.G., Eds.; CRC Press, Boca Raton Taylor & Francis Group, LLC: Boca Raton, FL, USA, 2006; pp. 863–869.
32. Olsen, S.R.; Sommers, L.E. Phosphorus. In *Methods of Soil Analysis*; Miller, A.L., Keeney, D.R., Eds.; American Society of Agronomy Madison: Madison, WI, USA, 1982; pp. 403–427.
33. Brookes, P.C.; Powlson, D.S.; Jenkinson, D.S. Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* **1982**, *14*, 319–329. [[CrossRef](#)]
34. Wu, J.; He, Z.; Wei, W.; O'Donnell, A.G.; Syers, J.K. Quantifying microbial biomass phosphorus in acid soils. *Biol. Fertil. Soils* **2000**, *32*, 500–507. [[CrossRef](#)]
35. Cleveland, C.C.; Liptzin, D. C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* **2007**, *85*, 235–252. [[CrossRef](#)]
36. Tian, H.Q.; Chen, G.S.; Zhang, C.; Melillo, J.M.; Hall, C.A.S. Pattern and variation of C:N:P ratios in China's soils: A synthesis of observational data. *Biogeochemistry* **2010**, *98*, 139–151. [[CrossRef](#)]

37. Zhou, J.; Wu, Y.H.; Prietzel, J.; Bing, H.J.; Yu, D.; Sun, S.Q.; Luo, J.; Sun, H.Y. Changes of soil phosphorus speciation along a 120-year soil chronosequence in the Hailuoguo Glacier retreat area (Gongga Mountain, SW China). *Geoderma* **2013**, *195*, 251–259. [[CrossRef](#)]
38. Guelland, K.; Hagedorn, F.; Smittenberg, R.H.; Goransson, H.; Bernasconi, S.M.; Hajdas, I.; Kretzschmar, R. Evolution of carbon fluxes during initial soil formation along the forefield of Damma glacier, Switzerland. *Biogeochemistry* **2013**, *113*, 545–561. [[CrossRef](#)]
39. Kumar, S.; Maiti, S.K.; Chaudhuri, S. Soil development in 2–21 years old coalmine reclaimed spoil with trees: A case study from Sonepur-Bazari opencast project, Raniganj Coalfield, India. *Ecol. Eng.* **2015**, *84*, 311–324. [[CrossRef](#)]
40. Yuan, Y.; Zhao, Z.Q.; Niu, S.Y.; Li, X.Z.; Wang, Y.Y.; Bai, Z.K. Reclamation promotes the succession of the soil and vegetation in opencast coal mine: A case study from Robinia pseudoacacia reclaimed forests, Pingshuo mine, China. *Catena* **2018**, *165*, 72–79. [[CrossRef](#)]
41. Batjes, N.H. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* **1996**, *47*, 151–163. [[CrossRef](#)]
42. Vitousek, P.M.; Porder, S.; Houlton, B.Z.; Chadwick, O.A. Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol. Appl.* **2010**, *20*, 5–15. [[CrossRef](#)]
43. Walker, T.W.; Syers, J.K. The fate of phosphorus during pedogenesis. *Geoderma* **1976**, *15*, 1–19. [[CrossRef](#)]
44. Crews, T.E.; Kurina, L.M.; Vitousek, P.M. Organic matter and nitrogen accumulation and nitrogen fixation during early ecosystem development in Hawaii. *Biogeochemistry* **2001**, *52*, 259–279. [[CrossRef](#)]
45. Zhou, J.; Bing, H.J.; Wu, Y.H.; Yang, Z.J.; Wang, J.P.; Sun, H.Y.; Luo, J.; Liang, J.H. Rapid weathering processes of a 120-year-old chronosequence in the Hailuoguo Glacier foreland, Mt. Gongga, SW China. *Geoderma* **2016**, *267*, 78–91. [[CrossRef](#)]
46. Wali, M.K. Ecological succession and the rehabilitation of disturbed terrestrial ecosystems. *Plant Soil* **1999**, *213*, 195–220. [[CrossRef](#)]
47. Pietrzykowski, M.; Krzaklewski, W. Soil organic matter, C and N accumulation during natural succession and reclamation in an opencast sand quarry (southern Poland). *Arch. Agron. Soil Sci.* **2007**, *53*, 473–483. [[CrossRef](#)]
48. Jencks, E.; Tyron, E.; Contri, M. Accumulation of nitrogen in minesoils seeded to black locust 1. *Soil Sci. Soc. Am. J.* **1982**, *46*, 1290–1293. [[CrossRef](#)]
49. Recous, S.; Mary, B.; Faurie, G. Microbial immobilization of ammonium and nitrate in cultivated soils. *Soil Biol. Biochem.* **1990**, *22*, 913–922. [[CrossRef](#)]
50. Singh, A.N.; Raghubanshi, A.S.; Singh, J.S. Impact of native tree plantations on mine spoil in a dry tropical environment. *For. Ecol. Manag.* **2004**, *187*, 49–60. [[CrossRef](#)]
51. Egli, M.; Fitze, P.; Mirabella, A. Weathering and evolution of soils formed on granitic, glacial deposits: Results from chronosequences of Swiss alpine environments. *Catena* **2001**, *45*, 19–47. [[CrossRef](#)]
52. Burga, C.A.; Krusi, B.; Egli, M.; Wernli, M.; Elsener, S.; Zieffle, M.; Fischer, T.; Mavris, C. Plant succession and soil development on the foreland of the Morteratsch glacier (Pontresina, Switzerland): Straight forward or chaotic? *Flora* **2010**, *205*, 561–576. [[CrossRef](#)]
53. Dümig, A.; Smittenberg, R.; Kogel-Knabner, I. Concurrent evolution of organic and mineral components during initial soil development after retreat of the Damma glacier, Switzerland. *Geoderma* **2011**, *163*, 83–94. [[CrossRef](#)]
54. Anderson, J.P.E.; Domsch, K.H. Quantities of plant nutrients in the microbial biomass of selected soils. *Soil Sci.* **1980**, *130*, 211–216. [[CrossRef](#)]
55. Azam, F.; Yousaf, M.; Hussain, F.; Malik, K.A. Determination of biomass-N in some agricultural soils of Punjab, Pakistan. *Plant Soil* **1989**, *113*, 223–228. [[CrossRef](#)]
56. Yu, Z.Y.; Chen, F.S.; Zeng, D.H.; Zhao, Q.; Chen, G.S. Soil inorganic nitrogen and microbial biomass carbon and nitrogen under pine plantations in zhanggutai sandy soil. *Pedosphere* **2008**, *18*, 775–784. [[CrossRef](#)]
57. Asensio, V.; Covelo, E.F.; Kandeler, E. Soil management of copper mine tailing soils—Sludge amendment and tree vegetation could improve biological soil quality. *Sci. Total Environ.* **2013**, *456*, 82–90. [[CrossRef](#)] [[PubMed](#)]

58. Anderson, J.D.; Ingram, L.J.; Stahl, P.D. Influence of reclamation management practices on microbial biomass carbon and soil organic carbon accumulation in semiarid mined lands of Wyoming. *Appl. Soil Ecol.* **2008**, *40*, 387–397. [[CrossRef](#)]
59. Banning, N.C.; Grant, C.D.; Jones, D.L.; Murphy, D.V. Recovery of soil organic matter, organic matter turnover and nitrogen cycling in a post-mining forest rehabilitation chronosequence. *Soil Biol. Biochem.* **2008**, *40*, 2021–2031. [[CrossRef](#)]

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