Effects of Mixing Feldspathic Sandstone and Sand on Soil Microbial Biomass and Extracellular Enzyme Activities—A Case Study in Mu Us Sandy Land in China

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Abstract: Microbial biomass, extracellular enzyme activity, and their stoichiometry in soil play an important role in ecosystem dynamics and functioning. To better understand the improvement of sand soil quality and the limitation of soil nutrients after adding feldspathic sandstone, we investigated changes in soil microbial activity after 10 months of mixing feldspathic sandstone and sand, and compared the dynamics with soil properties. We used fumigation extraction to determine soil microbial biomass carbon (MBC), nitrogen (MBN), phosphorus (MBP), and microplate fluorometric techniques to measure soil β-1,4-glucosidase (BG), β-1,4-xylosidase (BX), β-D-cellobiohydrolase (CBH), N-acetyl-β-glucosaminidase (NAG), and Alkaline phosphatase (AKP). We also measured soil organic carbon (SOC), pH, electrical conductivity (EC), soil inorganic carbon (SIC), and soil water content (SWC). Our results showed that the soil microbial biomass C, N, P, and individual extracellular enzyme activities significantly increased in mixed soil. Similarly, the soil microbial biomass C:N, C:P, N:P, MBC:SOC, and BG:NAG significantly increased by 54.3%, 106.3%, 33.1%, 23.0%, and 65.4%, respectively. However, BG:AKP and NAG:AKP decreased by 19.0% and 50.3%, respectively. Additionally, redundancy analysis (RDA) and Pearson’s correlation analysis showed that SWC, SOC, porosity and field capacity were significantly associated with soil microbial biomass indices (i.e., C, N, P, C:N, C:P, N:P in microbial biomass, and MBC:SOC) and extracellular enzyme activity metrics (i.e., individual enzyme activity, ecoenzymatic stoichiometry, and vector characteristics of enzyme activity), while pH, EC, and SIC had no correlation with these indices and metrics. These results indicated that mixing feldspathic sandstone and sand is highly susceptible to changes in soil microbial activity, and the soil N limitation decreased while P became more limited. In summary, our research showed that adding feldspathic sandstone into sand can significantly improve soil quality and provide a theoretical basis for the development of desertified land resources.

Keywords: feldspathic sandstone; sand soil; Mu Us sandy land; soil microbial biomass; extracellular enzyme activity; ecological stoichiometry
1. Introduction

Desertification is a serious ecological and environmental problem faced by China and even other countries in the world, affecting about one-sixth of the world’s population [1]. Desertification is of grave concern because of its threats to human survival and societal development [2]. Drylands cover more than one-third of the Earth’s continental surface and thus constitute the most extensive terrestrial biome on the planet [3]. Carbon storage in drylands account for 36% of the total global carbon storage [4]. Arid ecosystems are often characterized by low energy and nutrient availability of soil microbes and the availability of N and P generally limits primary productivity and microbial activity. Research on the quality of desert soil has recently changed focus from the basic physical and chemical properties of soil and the sand-fixing mechanism of sandy plants to soil microbial characteristics [5,6]. It is very important to study soil microbes in desertified and arid areas, especially after adding feldspathic sandstone into sand so that we may better understand if and how desertification can be reversed.

Changes in land conditions inevitably alter soil microbial metabolism and energy flow [7,8]. Studying the soil microbial biomass carbon (MBC), nitrogen (MBN), phosphorus (MBP) and its stoichiometry after adding feldspathic sandstone into sand can inform us about how soil nutrients are transformed. For example, Chen et al. [9] proved that the change of MBC:MBN was mainly related to abiotic variables, while the changes of MBC:MBP and MBN:MBP were mainly related to changes in soil microbial community structure. Thus, soil microbial communities respond differently to soil resources. Additionally, Zhao et al. [10] reported that MBC:SOC increased as afforested lands aged. This indicates that soil microbial stoichiometry can adaptively change with resource stoichiometry [11], and that soil microbial quotient can reflect stress effects and can be used as ecological physiological indicators. Similarly, Paul and Clark [12] suggested that higher MBC:MBN may reflect a larger overall investment in C-rich structural cellular material, while Elser et al. [13] suggested that lower MBN:MBP may reflect the higher allocation to P-rich ribosomes. Therefore, it is necessary to investigate the relationship between soil nutrient resources and microbial biomass, especially the ratio of microbial biomass C:N:P after mixing feldspathic sandstone and sand.

Recently, the most widely measured and extensively studied extracellular enzyme activities have included β-1,4-glucosidase (BG), cellobiohydrolase (CBH), and β-D-xylopyranoside (BX), indicators of energy (C) demand, an indicator of N demand known as β-1,4-N-acetylglucosaminidase (NAG), acid or alkaline phosphatase (AP or AKP), and indicators of P demand [3,14,15]. The potential activity of these extracellular enzymes can reflect the biogeochemical equilibrium between nutrient requirements of microbial assemblages and environmental nutrient availability [16–18]. For example, Chapin et al. [19] suggested that soil microbes would secrete more AP enzymes to meet the P demand in soils with low P availability. Tapia-Torres et al. [3] found that lower BG:NAG and MBC:MBN ratios indicated greater soil microbial demand for N versus C. Peng et al. [20] reported that BG:AP and (NAG + LAP):AP of typical steppes and desert steppes were lower than those of meadow steppes, implying that typical steppes and desert steppes had stronger P limitations. Therefore, studying the soil extracellular enzymes and their stoichiometry can provide an important step in assessing the stoichiometry and energy limitation of soil microbial metabolism after mixing feldspathic sandstone and sand.

As one of four large sandy lands in China, the Mu Us Sandy Land has a fragile ecological environment and is one of the most severe desertification areas in northern China [2]. This area is not only threatened by desertification or soil degradation, but is also characterized by feldspathic sandstone and sand with loose and easily weathered structure [2,6]. The feldspathic sandstone has low diagenesis, is hard as stone without water, and is soft as mud when wet. It has good water retention capacity. However, sand has small porosity, poor water-holding capacity, low structural strength, and leaks water and fertilizer. The sandstone and sand are known as the local “two harms”. It would be great if we could use their complementarity in texture and permeability to improve the local soil quality. Based on these sandy characteristics, we proposed to improve soil quality by mixing feldspathic sandstone and sand to support local agriculture and gradually form new soil resources. This strategy can avoid the pollution of chemical residues or artificial water retaining agents and save costs, thus
turning the "two harms" into "one treasure". Thus, we selected this area to study soil microbial biomass and extracellular enzyme activity and their stoichiometry in sand vs. a mix of sand and feldspathic sandstone, thereby providing a theoretical basis and technical guidance for global soil desertification control. We hypothesized that: (i) soil microbial biomass, and extracellular enzyme activity increased in mixed soil, and (ii) soil organic carbon (SOC) and soil water content (SWC) were important drivers of soil microbial biomass and extracellular enzyme activity and their stoichiometry. Our objectives in this study were to: (i) assess changes in microbial biomass and enzyme activity after adding feldspathic sandstone into sand, (ii) characterize the changes in microbial biomass stoichiometric ratios (MBC:MBN, MBC:MBP, and MBN:MBP) and extracellular enzyme activity stoichiometric ratio (BG:NAG, BG:AKP, and NAG:AKP), and (iii) explore the relationship between soil basic properties, soil microbial biomass, soil enzyme activity, and soil stoichiometry.

2. Materials and Methods

2.1. Study Area Description

Mu Us Sandy Land (37°27′–39°22′N, 107°20′–111°30′E) is a famous desertified area in China. It covers an area of approximately 40,000 km² in the southern part of Ordos City in the Inner Mongolia Autonomous Region, the northern part of Yulin City in Shaanxi Province, and northeast part of Yanchi County in the Ningxia Hui Autonomous Region [21]. Our study area is in the southern edge of the Mu Us Sandy Land, Yulin City in the northwest of Shaanxi Province, with an elevation of 1206~1215 m [5]. Wind erosion, water erosion, and desertification are all serious problems in this region. The annual average temperature is 8.1 °C, annual average precipitation is 250~440 mm, annual average evaporation is 1904 mm, and the average wind speed is 3.5 m/s in the region. It is in the typical semi-arid temperate semi-arid continental monsoon climate zone and is an ecologically fragile agriculture-pasture ecotone. The sand ridges in the area are mainly composed of red or gray Cretaceous feldspathic sandstone. Feldspathic sandstone and sand alternately distribute in Mu Us Sandy Land [22]. Aeolian sandy soil is the main soil type, accounting for 93.4% of the total area of Mu Us Sandy Land.

2.2. Experimental Design and Soil Sampling

In July 2018, we collected feldspathic sandstone and sand at 0–10 cm depth in the study area. Three replicate plots (20 × 20 m) were created within each site for sampling. In each plot, we used a soil auger with an inner diameter of 5 cm to collect 20 soil samples of the same depth in an “S” shape, and then uniformly mixed 20 replicate samples to constitute a single soil sample. Each sample was stored in a portable incubator equipped with ice packs and then immediately transported to the laboratory for the following processes: mixing the feldspathic sandstone and sand according to the mass ratio of 1:5 and put it into a 30 × 30 × 30 cm container, while the unmixed undisturbed sand was used as control. Each treatment was repeated three times and a total of six containers were laid. In order to simulate the land condition of the sand mixed with feldspathic sandstone in the Mu Us Sandy Land, we placed the containers in the field scientific experimental station in Mu Us Sandy Land until May 2019. The sand and mixed soil samples were incubated for 10 months and then taken out. Each sample was sieved through a 2 mm aperture and then divided into four parts. The first part was used to determine the SWC; the second part was stored in a 4 °C refrigerator for microbial biomass analysis; the third part was stored in the refrigerator at −20 °C for subsequent determination of soil extracellular enzyme activity; and the remaining part was air-dried at room temperature to determine the basic physical and chemical properties of the soil.

2.3. Analysis of Soil Physicochemical Properties and Microbial Biomass

The SWC was measured by an oven-dry method at 105 °C and expressed as dry mass. Soil pH was determined in a 1:2.5 soil:water suspension using an automated acid-base titrator (Metrohm 702), and soil conductivity (EC) was determined using a conductivity meter. Soil bulk density (BD) was
determined using the ring knife method. Soil texture was measured using a laser particle size analyzer (Mastersizer 2000, Malvern, UK) [20]. SOC was determined using the Walkley-Black method [23]. Soil total carbon (STC) content was determined using an elemental analyzer (Vario MACRO cube CN; Germany) [24]. Soil inorganic C (SIC) content was difference between STC and SOC. Soil field capacity was determined by the ring knife method. Soil porosity was performed as described by Sun et al. [25]. Soil microbial biomass C, N, and P were estimated using chloroform fumigation-K$_2$SO$_4$ extraction and potassium persulfate digestion methods [26–28]. The fumigated and non-fumigated soil samples were extracted with 0.5M potassium sulfate for 30 min, and the measurements of MBC and MBN were determined using a TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan). The MBP was measured photometrically with a continuous flow auto analyzer. The percentage of SOC present as MBC was calculated as a microbial quotient (MBC:SOC). The ratio of microbial biomass C, N, P were calculated as microbial biomass stoichiometry (MBC:MBN, MBC:MBP, and MBN:MBP) [29].

2.4. Analysis of Soil Extracellular Enzyme Activities

In this experiment, five soil extracellular enzyme activities were determined according to a modified version of the standard fluorometric techniques [20]. The functions of different soil extracellular enzymes are shown in Table 1. Briefly, the principle of enzyme activity determination is that the enzymes hydrolyze the artificial substrates and produce 4-Methylumbelliferone (MUB), which could be determined by fluorescence [30]. The specific measurement steps were as follows: 1 ± 0.01 g of fresh soil was weighed into a 250 mL plastic bottle, and 125 mL of deionized water was added, then the soil particles were all broken by shaking on a rotary shaker. Then, we added 200 µL of soil suspension liquid and 50 µL of substrate (200 µmol·L$^{-1}$) to the microplates (6 replicates per sample), and a blank control was added with 200 µL of soil suspension liquid and 50 µL of deionized water. The microplates were then incubated for 4 h at 25 ºC in the dark. Finally, 10µL of NaOH (concentration: 0.5 mol·L$^{-1}$) was added to each sample well to terminate the reaction. The fluorescence value was measured using a microplate reader after 1 min. After correcting for negative controls and quenching, all activities were expressed in units of nmol·g$^{-1}$·h$^{-1}$.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC</th>
<th>Abbr.</th>
<th>Substrate</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-1,4-glucosidase</td>
<td>EC 3.2.1.21</td>
<td>BG</td>
<td>4-MUB-β-D-glucoside</td>
<td>Hydrolysis of cellobiose to glucose during carbon cycling</td>
</tr>
<tr>
<td>β-1,4-xylosidase</td>
<td>EC 3.2.1.37</td>
<td>BX</td>
<td>4-MUB-β-D-xylopyranoside</td>
<td>Hydrolysis of cellulose to form hemicellulose during carbon cycle</td>
</tr>
<tr>
<td>Celllobiohydrolase</td>
<td>EC 3.2.1.91</td>
<td>CBH</td>
<td>4-MUB-β-D-celllobioside</td>
<td>Hydrolysis of cellulose to produce sucrose during carbon cycle</td>
</tr>
<tr>
<td>β-N-acetylglosaminidase</td>
<td>EC 3.2.1.14</td>
<td>NAG</td>
<td>4-MUB-N-acetyl-β-D-glucosaminide</td>
<td>Participation in the hydrolysis of chitin during the carbon-nitrogen cycle</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>EC 3.1.3.1</td>
<td>AKP</td>
<td>4-MUB-phosphate</td>
<td>Hydrolizes phosphates from phosphosaccharides and phospholipids during the phosphorus cycle</td>
</tr>
</tbody>
</table>

EC: enzyme commission classification; MUB: methylumbelliferyl.

The reason for selecting the five soil extracellular enzymes is that their potential activities are usually related to the rate of microbial metabolism and biogeochemical processes. Among them, BG, NAG, and AKP are commonly used as indicators of microbial nutrient demand [16] and were also used as the indicators of C, N, and P acquiring enzymes in previous studies about soil extracellular enzyme stoichiometry [10,15]. Because the other two major C-acquiring enzymes (CBH and BX) were much lower than the activity of BG, they had no effect on the soil extracellular enzyme stoichiometry results. In addition, vector analysis (Vector length, VL; Vector angle, VA) of soil extracellular enzyme stoichiometry is a means for visualizing the relative C, N, and P controls on soil microbial communities, which can reflect soil nutrient limitations [31]. Our analysis of the enzyme activity vectors clearly showed the changes in relative resource demands caused by decomposer communities during the
decomposition process, which cannot be observed by alternative analyses [32]. This method is thus considered to compensate for the fact that pairwise comparisons of enzyme activities cannot not reveal resource co-limitations [33]. Therefore, in our experiments, this method was used to better quantify and visualize the relative C, N, and P controls on soil microbial communities:

\[
\text{Vector length} = \sqrt{\left(\frac{\text{BG}}{\text{BG} + \text{AKP}}\right)^2 + \left(\frac{\text{BG}}{\text{BG} + \text{NAG}}\right)^2}
\]

\[
\text{Vector angle} = \text{DEGREES}\left(\text{ATAN2}\left(\frac{\text{BG}}{\text{BG} + \text{AKP}}, \frac{\text{BG}}{\text{BG} + \text{NAG}}\right)\right).
\]

The relative vector length quantifies relative C vs. nutrient limitation and the vector angle quantifies the relative P vs. N limitation. The longer the vector length, the larger the C limitation, and the larger the vector angle, the stronger the P limitation [33].

2.5. Statistical Analyses

Excel 2013 and SPSS.22 were used for pre-processing and statistical analysis of the data. All results were reported as means ± standard errors (SE) for the three replicates. Graphing was performed using Origin 8.0 software. We used SPSS.22 to test the normality and variance homogeneity of the data (Table S1). One-way ANOVA was used to compare the significant changes in soil characteristics, soil microbial biomass and extracellular enzyme activities in sand vs. a mix of sand and feldspathic sandstone. We calculated the MBC:MBN, MBC:MBP, MBN:MBP, MBC:SOC, BG:NAG, BG:AKP, NAG:AKP, BG/(BG + AKP), BG/(BG + NAG), VL, and VA for all cases. These indices, as measures of the resources directed towards the acquisition of organic P and organic N relative to C, were used to test for the soil extracellular enzyme distribution across ecosystems and to compare the relative nutrient demand between the two soils. The relationships between soil extracellular enzyme indices and edaphic abiotic and biotic factors were determined using Pearson’s correlation analysis (SPSS.22). Redundancy analysis (RDA) was used to identify the interrelationships between soil physicochemical properties and soil microbial biomass. The RDA was carried out using the CANOCO 4.5 software package. All the results were considered statistically significant at \( P < 0.05 \).

3. Results

3.1. Effects of Mixing Feldspathic Sandstone and Sand on Soil Physicochemical Properties

Table 2 summarizes the basic physicochemical properties of soil in sand vs. a mix of sand and feldspathic sandstone. In sand soil, the soil texture type was sand, but the soil texture type was transformed into sandy loam in mixed soil. Soil pH in both soils was weakly alkaline and was slightly lower in mixed soil than in sand soil. Conversely, soil EC in mixed soil was more than 10.8% higher than sand soil albeit not significant between the two types of soils. Compared with sand soil, the SWC, STC, SOC, porosity, and field capacity in mixed soil significantly increased by 73.3%, 101.9, 163.2%, 32.2%, and 140.0%, respectively; whereas the BD and SIC decreased by 9.2% and 16.4%, respectively.

3.2. Effects of Mixing Feldspathic Sandstone and Sand on Soil Microbial Biomass Indices

Figure 1 shows that soil MBC, MBN, MBP, MBC:MBN, MBC:MBP, MBN:MBP, and MBC:SOC were present in significantly higher quantities in mixed soil than in sand soil by 222.8%, 112.0%, 58.2%, 54.3%, 106.3%, 33.1%, and 23.0%, respectively. Notably, changes in the MBC, MBN, MBP, MBC:MBP, and MBC:MBN varied more than changes in MBN:MBP and MBC:SOC.
Soil microbial biomass (mg/kg) was determined in sand soil, and we found the BG:AKP and NAG:AKP ratios showed significant downward trends, decreasing by 19.0% and 54.3%, respectively. Values are expressed as mean ± SE, n = 3. * and ** indicate statistical significance at the 0.05 and 0.01 probability levels, respectively. EC, BD, STC, SOC, SIC, and SWC are the abbreviations of soil electrical conductivity, soil bulk density, soil total carbon, soil organic carbon, soil inorganic carbon, and soil water content, respectively.

3.3. Effects of Mixing Feldspathic Sandstone and Sand on Soil Extracellular Enzymes Activities and Its Stoichiometry

For individual enzymes, the values of BG, NAG, AKP, CBH, and BX in mixed soil were 2.4, 1.0, 3.1, 1.5, and 3.0 times higher than those in sand soil, respectively (Figure 2). For the ecoenzymatic stoichiometry, the BG:NAG ratio increased significantly in mixed soil, with an increase of 65.4%. However, the BG:AKP and NAG:AKP ratios showed significant downward trends, decreasing by 19.0% and 50.3%, respectively (Figure 2). In addition, a quantitative vector analysis method for estimating resource allocation strategies of microbial communities was shown visually in Table 3. Compared with sand soil, we found the BG/(BG + AKP) was lower, while the BG/(BG + NAG), vector length, and vector angle were higher in mixed soil. Notably, all vector characteristics of enzyme activity had significant differences between two soils.

Table 2. Soil characteristics in sand vs. a mix of sand and feldspathic sandstone.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sand Soil</th>
<th>Mixed Soil</th>
<th>F (1, 5)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td>sand</td>
<td>sandy loam</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>pH</td>
<td>8.34 ± 0.07</td>
<td>8.16 ± 0.17</td>
<td>1.07</td>
<td>0.36</td>
</tr>
<tr>
<td>EC (µs/cm)</td>
<td>181.73 ± 14.98</td>
<td>201.37 ± 35.52</td>
<td>0.26</td>
<td>0.64</td>
</tr>
<tr>
<td>BD (g/cm³)</td>
<td>1.63 ± 0.05</td>
<td>1.47 ± 0.02</td>
<td>9.13</td>
<td>0.04 *</td>
</tr>
<tr>
<td>SWC (%)</td>
<td>2.66 ± 0.08</td>
<td>4.61 ± 0.04</td>
<td>479.31</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>STC (g/kg)</td>
<td>1.61 ± 0.21</td>
<td>3.25 ± 0.24</td>
<td>26.17</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>SOC (g/kg)</td>
<td>1.06 ± 0.09</td>
<td>2.79 ± 0.06</td>
<td>252.95</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>SIC (g/kg)</td>
<td>0.55 ± 0.18</td>
<td>0.46 ± 0.18</td>
<td>0.13</td>
<td>0.74</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>25.31</td>
<td>33.46</td>
<td>17.03</td>
<td>0.02 *</td>
</tr>
<tr>
<td>Field capacity (%)</td>
<td>6.88</td>
<td>16.51</td>
<td>216.39</td>
<td>&lt;0.01 **</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE, n = 3. * and ** indicate statistical significance at the 0.05 and 0.01 probability levels, respectively. EC, BD, STC, SOC, SIC, and SWC are the abbreviations of soil electrical conductivity, soil bulk density, soil total carbon, soil organic carbon, soil inorganic carbon, and soil water content, respectively.

Figure 1. Soil microbial biomass and microbial biomass C:N:P stoichiometry and microbial quotient in sand vs. a mix of sand and feldspathic sandstone. * and ** indicate statistical significance at the 0.05 and 0.01 probability levels, respectively. Error bars are the standard errors (n = 3).
Robustal biomass indices, but only microbial biomass indices. Microbial biomass C, N, P, microbial biomass C:N:P stoichiometry, and microbial quotient were examined using RDA and Pearson’s correlation analysis (Figure 3, Table S2). The results showed that SWC were significantly and positively correlated with all soil microbial biomass indices. STC and SOC showed significant positive correlations with MBC, MBN, MBP, MBC:MBN, MBC:MBP, and MBN:MBP, but no significant correlations with MBC:SOC. Furthermore, BD was negatively correlated with all microbial biomass indices, but only closely related to the changes in MBC, MBC:MBN, and MBC:SOC. In particular, pH and SIC had no significant effect on all soil microbial biomass indices. Altogether, SWC, STC, and SOC had major effects on soil microbial biomass indices, and BD had minor effects on soil microbial biomass indices, while pH, EC, and SIC had no effects on all soil microbial biomass indices.

**Table 3.** The vector characteristics of enzyme activity in sand vs. a mix of sand and feldspathic sandstone.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Sand Soil</th>
<th>Mixed Soil</th>
<th>$F_{(1, 5)}$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG/(BG + AKP)</td>
<td>0.302 ± 0.001</td>
<td>0.262 ± 0.005</td>
<td>67.09</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>BG/(BG + NAG)</td>
<td>0.85 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>116.41</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>Vector length</td>
<td>0.90 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>39.91</td>
<td>&lt;0.01 **</td>
</tr>
<tr>
<td>Vector angle</td>
<td>70.41 ± 0.06</td>
<td>73.82 ± 0.20</td>
<td>278.87</td>
<td>&lt;0.01 **</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE, $n = 3$. All samples included the values for β-1,4-glucosidase (BG), β-N-acetylglucosaminidase (NAG), and alkaline phosphatase (AKP). Vector angles in degrees, all other vector characteristics unitless. Calculations for vector length and vector angle described in the main text. ** means that the differences between the two lands for a metric is significant at the 0.01 level.

### 3.4. Relationships among Soil Physiochemical Properties, Microbial Biomass and Enzyme Activity

The relationships between soil physiochemical properties and soil microbial biomass indices (i.e., microbial biomass C, N, P, microbial biomass C:N:P stoichiometry, and microbial quotient) were examined using RDA and Pearson’s correlation analysis (Figure 3, Table S2). The results showed that SWC were significantly and positively correlated with all soil microbial biomass indices. STC and SOC showed significant positive correlations with MBC, MBN, MBP, MBC:MBN, MBC:MBP, and MBN:MBP, but no significant correlations with MBC:SOC. Furthermore, BD was negatively correlated with all microbial biomass indices, but only closely related to the changes in MBC, MBC:MBN, and MBC:SOC. In particular, pH and SIC had no significant effect on all soil microbial biomass indices. Altogether, SWC, STC, and SOC had major effects on soil microbial biomass indices, and BD had minor effects on soil microbial biomass indices, while pH, EC, and SIC had no effects on all soil microbial biomass indices.
Figure 3. Ordination plots of the results from the redundancy analysis to identify the relationships between soil physiochemical properties (red arrows), soil microbial biomass, microbial biomass C:N:P stoichiometry, and microbial quotient (blue arrows) \( (n = 3) \). The STC was highly correlated with SOC (Pearson R = 0.955), thus it was removed from this analysis (Table S2).

In addition, Pearson’s correlation coefficients showed a significant correlation between soil extracellular enzymes activities, ecoenzymatic stoichiometry, and vector characteristics of enzyme activity with edaphic abiotic and biotic factors (Table 4). The activities of all individual enzymes had closely relationship to SWC, STC, SOC, porosity, field capacity, MBC, MBN, MBP, MBC:MBN, and MBC:MBP, but had little relationship to the pH, EC, or SIC. In detail, SWC, STC, SOC, porosity, field capacity, MBC, MBN, MBP, MBC:MBN, and MBC:MBP showed a significant positive correlation with all individual enzyme activities. Conversely, CBH had no significant correlation with MBN:MBP and MBC:SOC, and BX was not closely related to MBC:SOC. Additionally, the activities of BG, AKP, and BX were also closely related to BD (negative correlation). For the ecoenzymatic stoichiometry and vector characteristics of enzyme activity, they were all significantly associated with all edaphic biotic factors except MBC:SOC, but only with some edaphic abiotic factors (e.g., BD, SWC, STC, SOC, porosity, and field capacity). Like individual enzymes, pH, EC, and SIC had no significant effect on the ecoenzymatic stoichiometry and vector characteristics of enzyme activity. Interestingly, SWC, STC SOC, porosity, and field capacity showed significant negative correlations with BG:AKP, NAG:AKP, and BG/(BG + AKP), but showed significant positive correlations with other enzyme activity metrics (i.e., individual enzyme activity, ecoenzymatic stoichiometry, and vector characteristics of enzyme activity).

Table 4. Pearson’s correlation coefficients \( (r) \) relating soil extracellular enzymes activities, ecoenzymatic stoichiometry and vector characteristics of enzyme activity with (a) edaphic abiotic factors (pH, EC, BD, SWC, STC, SOC, SIC, FC) and (b) biotic factors (MBC, MBN, MBP, MBC: MBN, MBC: MBP, and MBN: MBP) \( (n = 3) \).
Table 4. Cont.

<table>
<thead>
<tr>
<th>Edaphic Biotic Factors</th>
<th>MBC</th>
<th>MBN</th>
<th>MBP</th>
<th>MBC:MBN</th>
<th>MBC:MBP</th>
<th>MBN:MBP</th>
<th>MBC:SOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG</td>
<td>0.996 **</td>
<td>0.986 **</td>
<td>0.921 **</td>
<td>0.986 **</td>
<td>0.983 **</td>
<td>0.884 *</td>
<td>0.794</td>
</tr>
<tr>
<td>NAG</td>
<td>0.988 **</td>
<td>0.992 **</td>
<td>0.942 **</td>
<td>0.955 **</td>
<td>0.951 **</td>
<td>0.864 *</td>
<td>0.818 *</td>
</tr>
<tr>
<td>AKP</td>
<td>0.999 **</td>
<td>0.993 **</td>
<td>0.938 **</td>
<td>0.982 **</td>
<td>0.975 **</td>
<td>0.875 *</td>
<td>0.812 *</td>
</tr>
<tr>
<td>CBH</td>
<td>0.903 *</td>
<td>0.924 **</td>
<td>0.930 **</td>
<td>0.844 *</td>
<td>0.815 *</td>
<td>0.731</td>
<td>0.725</td>
</tr>
<tr>
<td>BX</td>
<td>0.985 **</td>
<td>0.971 **</td>
<td>0.896 *</td>
<td>0.984 **</td>
<td>0.982 **</td>
<td>0.866 *</td>
<td>0.806</td>
</tr>
<tr>
<td>BG:NAG</td>
<td>-0.979 **</td>
<td>-0.988 **</td>
<td>-0.952 **</td>
<td>-0.940 **</td>
<td>-0.922 **</td>
<td>-0.839 *</td>
<td>-0.822 *</td>
</tr>
<tr>
<td>BG:AKP</td>
<td>-0.966 **</td>
<td>-0.986 **</td>
<td>-0.928 **</td>
<td>-0.985 **</td>
<td>-0.979 **</td>
<td>-0.876 *</td>
<td>-0.794</td>
</tr>
<tr>
<td>NAG:AKP</td>
<td>-0.977 **</td>
<td>-0.986 **</td>
<td>-0.953 **</td>
<td>-0.937 **</td>
<td>-0.923 **</td>
<td>-0.834 *</td>
<td>-0.824 *</td>
</tr>
<tr>
<td>BG(BG + AKP)</td>
<td>0.975 **</td>
<td>0.961 **</td>
<td>0.968 *</td>
<td>0.980 **</td>
<td>0.975 **</td>
<td>0.867 *</td>
<td>0.764</td>
</tr>
<tr>
<td>BG(BG + NAG)</td>
<td>0.945 **</td>
<td>0.921 **</td>
<td>0.849 *</td>
<td>0.960 **</td>
<td>0.957 **</td>
<td>0.847 *</td>
<td>0.720</td>
</tr>
<tr>
<td>Vector length</td>
<td>0.996 **</td>
<td>0.997 **</td>
<td>0.954 **</td>
<td>0.967 **</td>
<td>0.956 **</td>
<td>0.859 *</td>
<td>0.822 *</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

EC, BD, STC, SOC, SIC, SWC, FC, MBC, MBN, and MBP are the abbreviations of soil electrical conductivity, soil bulk density, soil total carbon, soil organic carbon, soil inorganic carbon, soil water content, field capacity, microbial biomass carbon, microbial biomass nitrogen, and microbial biomass phosphorus, respectively.

4. Discussion

4.1. Explaining Variance in Soil Physiochemical Properties in Sand vs. a Mix of Sand and Feldspatic Sandstone

Soil texture is of great significance for regional soil improvement because it affects various soil traits, such as permeability, water holding capacity, arability, and nutrient migration, and the distribution and utilization efficiency in soil [34]. Our results showed that after 10 months of feldspatic sandstone and sand mixing, the soil texture transformed from sand to sandy loam, thus transforming the aeolian sandy soil (the sand soil) that was prone to leaking water and nutrients, severe wind and water erosion, and oligotrophs into a relatively stable soil with higher porosity and better field capacity. To some extent, the mixed soil appropriately made up the shortage of aeolian sandy soil, indicating that adding feldspatic sandstone into sand can improve the soil conditions. Organic matter is an important cementing material in the formation of soil aggregates, and its content is positively correlated with the stability and quantity of soil water–stable aggregates [35]. In our study, STC, SOC, SWC, and field capacity significantly increased, while SIC decreased in mixed soil, which not only indicated that mixing feldspatic sandstone and sand has obvious effects on carbon sequestration and water and fertility preservation, but also further indicated the soil structure was more stable in mixed soil. In general, mixing feldspatic sandstone and sand can reduce or prevent the leakage of water in the aeolian sandy soil, improve fertility, and weaken the hardening of feldspatic sandstone, thus achieving the dual purposes of controlling sand and improving feldspatic sandstone.

4.2. Explaining Variance in Soil Microbial Biomass Indices in Sand vs. a Mix of Sand and Feldspatic Sandstone

In our study, the microbial biomass of sand soil was consistent with that of desert biomes reported by Xu et al. [36]. However, the soil microbial biomass significantly increased in mixed soil (Figure 2). This is because that adding feldspatic sandstone into sand for 10 months significantly improved soil physiochemical properties, provided abundant nutrients such as carbon, nitrogen, and phosphorus for microbes, promoted microbial reproduction, and thus helped to increase soil microbial biomass C, N, and P [37]. The increase in microbial biomass also indicates that soil microbes have a significant improve in the ability to fix soil carbon, nitrogen, and phosphorus [38]. At the same time, the addition of feldspatic sandstone increases the porosity of the original sandy soil, meaning better soil aeration, which further enhances soil microbial growth and activity [39]. Moreover, we found a significant positive correlation between microbial biomass C, N, P and SWC, STC, SOC, field capacity (Figure 3, Table S2), which is consistent with previous studies [40–43], indicating that microbial biomass can characterize soil fertility status and water capacity. We also observed that soil MBN and MBP were
significantly positively correlated with MBC, indicating that MBC accumulation promoted the fixation of microbial to N and P nutrients, while the biomineralization and consumption of N and P in soil depended on the transformation and decomposition of organic matter by soil microbes [31]. These results also indicated that there was a good mutual constraint between the microbial biomass C, N, and P [36,44].

In addition, the ecological stoichiometry of soil microbial biomass is of great significance for exploring the restricted nutrients of soil ecosystems. Xu et al. [36] reported that the global average soil MBC:MBN, MBC:MBP, and MBN:MBP ratios were 7.6, 42.4, and 5.6, respectively. However, in our study the soil MBC:MBP and MBN:MBP in sand vs. a mix of sand and feldspathic sandstone were lower than the global research results, which may be related to soil P infertility in the study area [44]. Simultaneously, soil MBC:MBN, MBC:MBP, and MBN:MBP increased in mixed soil. The first possible explanation for this observation is that MBC was more sensitive to environmental changes than MBN and MBP [45]. The second possible explanation is related to the increase in biodiversity and the change in soil properties after adding feldspathic sandstone. The increase in soil MBC:MBP may have also been due to the relative decrease in the demand for P-rich ribosomal RNA by microbial growth [13]. Likewise, microbes can take up excess resources and store them in the form of glycogen or polyphosphates, thereby inducing changes in MBC:MBN ratio [46]. Additionally, Anderson and Domsch [47] pointed out that MBC:SOC varied between 0.27% and 7.0%. However, the variation range of MBC:SOC was 7.35%–10.45% in our study, which was higher than previous studies. This difference may be because SOC was poor in our study area, and the microbial metabolic cycle is short. In order to maintain soil nutrient content, the proportion of MBC in SOC must be increased to maintain high organic matter metabolism and nutrient cycling [47]. We also found that the MBC:SOC in mixed soil was higher than in sand soil. This may be because the turnover rate in MBC after adding feldspathic sandstone is higher than that in soil C. Another possible explanation is that the quantity, quality, and cycle of the organic matter input into the mixed soil were better than those in sand soil, resulting in higher soil microbial activity and microbial biomass and promoting the turnover and decomposition rate of soil organic matter. Additionally, Anderson and Domsch [47] pointed out that MBC:SOC varied between 0.27% and 7.0%. However, the variation range of MBC:SOC was 7.35%–10.45% in our study, which was higher than previous studies. This difference may be because SOC was poor in our study area, and the microbial metabolic cycle is short. In order to maintain soil nutrient content, the proportion of MBC in SOC must be increased to maintain high organic matter metabolism and nutrient cycling [47]. We also found that the MBC:SOC in mixed soil was higher than in sand soil. This may be because the turnover rate in MBC after adding feldspathic sandstone is higher than that in soil C. Another possible explanation is that the quantity, quality, and cycle of the organic matter input into the mixed soil were better than those in sand soil, resulting in higher soil microbial activity and microbial biomass and promoting the turnover and decomposition rate of soil organic matter. Moreover, it is generally accepted that soil nutrients are the basis of soil extracellular enzyme activity, and soil extracellular enzyme activity is the driving force of soil nutrient cycling, which are closely related to each other [53]. Therefore, it is not surprising that there is a significant correlation between the activity of five extracellular enzymes and SOC, microbial biomass C, N, and P, and its

4.3. Explaining Variance in Soil Extracellular Enzyme Activity Metrics in Sand vs. a Mix of Sand and Feldspathic Sandstone

Soil extracellular enzymes are very sensitive to changes in biotic and abiotic environments and are active participants in the material circulation and energy conversion of soil system, and are often regarded as important indicators for determining soil quality [31,49]. Studies have shown that soil extracellular enzymes are the proximate agents of nutrient decomposition [20]. Therefore, in our study, soil enzyme activity in mixed soil was significantly higher than that in sand soil, probably because more soil organic carbon enrichment after adding feldspathic sandstone for 10 months provided relatively abundant C sources for microbes [50,51]. This result may also be related to the increase of SWC [52]. Our results showed that there was a significant and positive correlation between the five soil extracellular enzyme activities (Table S3), indicating that the soil extracellular enzymes were interrelated and interact with each other, and there was a symbiotic relationship during the interaction process. Moreover, it is generally accepted that soil nutrients are the basis of soil extracellular enzyme activity, and soil extracellular enzyme activity is the driving force of soil nutrient cycling, which are closely related to each other [53]. Therefore, it is not surprising that there is a significant correlation between the activity of five extracellular enzymes and SOC, microbial biomass C, N, and P, and its
stoichiometry in our study (Table 4). It is implied that soil extracellular enzyme activity can be used as an important indicator to measure soil nutrient status [52].

Soil extracellular enzyme stoichiometry can reveal the energy limitations in microbial growth and metabolism and can be used to evaluate the demand of soil microbes for C, N, and P nutrient resources [14]. In our study, soil BG:NAG increased, whereas soil NAG:AP decreased in mixed soil. This suggests that the microbes in mixed soil reduced the production of N-acquired enzymes (NAG), so the soil N was relatively sufficient after adding feldspathic sandstone into sand. Similarly, a reduction in BG:AP and NAG:AP in mixed soil indicated that soil microbial growth was limited by P availability. Our findings agree with the resource allocation perspective that microbes are expected to allocate their resource reserves optimally toward acquisition of the most limiting resource [54]. It is worth noting that many studies have reported significant correlations between soil pH and enzyme activity and its stoichiometry [16,18,20,52,55]. However, there was no significant correlation between pH and enzyme activity in our study, which may be due to the absence of significant changes in soil pH in sand and mixed soil. Likewise, it is implied that pH is not the major driving factor affecting the change of enzyme activity after adding feldspathic sandstone into sand in this area. Intriguingly, we found that BG:AP and MBC:MBP, NAG:AP, and MBN:MBP both showed a significant negative correlation, while BG:NAG and MBC:MBN showed a significant positive correlation. These results might have been observed because adding feldspathic sandstone may alter the soil resource status, thereby regulating enzyme production soil microbial organisms [10], and indicates that the relative activity of enzymes can reflect the elemental composition of microbial biomass. We also observed that SWC had a significant positive correlation with BG:NAG, and had a significant negative correlation with NAG:AP. This result was not only supported by previous studies [3,20], but also proved by our observation that the increase of SWC in mixed soil caused a relative decrease in N-acquired enzyme activity, which further indicates that the soil N content improved after adding feldspathic sandstone.

Moreover, we found that the mixed soil had greater vector lengths, suggesting higher relative C limitation [31], consistent with the higher BG/(BG + NAG), but inconsistent with the lower BG/(BG + AKP). A possible reason that our result is contrary to this expectation is that the soil C limitation was not very severe and may be gradually reduced with the input of root exudation and litter after mixing feldspathic sandstone and sand [33]. The mixed soil also had a higher vector angle, indicating the stronger P limitation, coincident with the lower values of BG/(BG + AKP). Similarly, as with extracellular enzyme stoichiometry, enzyme activity vector lengths and angles as well as BG/(BG + AKP) and BG/(BG + NAG) were significantly associated with SWC, SOC, microbial biomass C, N, and P, and its stoichiometry. These findings also supported our hypothesis that SOC and SWC were important drivers of increased soil extracellular enzyme activity and its stoichiometry.

5. Conclusions

Our study showed the relative differences in soil microbial biomass and extracellular enzyme activities after mixing feldspathic sandstone and sand in Mu Us Sandy Land. The significant increase in soil microbial biomass and individual extracellular enzyme activity in mixed soil indicated that soil quality status improved after adding feldspathic sandstone into sand. Soil microbial biomass C:N:P, extracellular enzyme stoichiometry, and enzyme activity vector analysis showed that soil N was relatively abundant, but P became increasingly limiting after adding feldspathic sandstone. Moreover, Pearson’s correlation coefficients and RDA analysis indicated that soil microbial biomass, extracellular enzyme activities, and their stoichiometry were largely affected by SOC, SWC, porosity, and field capacity. Overall, our research showed that adding feldspathic sandstone into sand significantly altered soil microbial activity and soil nutrient resources. These factors, in turn, are key to determining the success of feldspathic sandstone in improving sand and ultimately changing the function of ecosystems in desertified areas.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/9/19/3963/s1, Table S1: Variance homogeneity test; Table S2: Pearson’s correlation coefficients (r) matrix with soil physiochemical
properties and microbial characteristics (i.e., microbial biomass, microbial biomass C:N:P stoichiometry, and microbial quotient); Table S3: Pearson’s correlation coefficients (r) matrix with soil extracellular enzymes activities, ecoenzymatic stoichiometry and enzyme activity metrics; Table S4: Basic properties of feldspathic sandstone.

**Author Contributions:** Writing-original draft, X.F.; investigation, L.Z.; supervision, F.Z. and H.B.; Writing-review and editing, R.D.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

- Mixed soil: a mix of sand and feldspathic sandstone
- RDA: redundancy analysis
- Soil characteristics
  - EC: soil electrical conductivity
  - BD: soil bulk density
  - STC: soil total carbon
  - SOC: soil organic carbon
  - SIC: soil inorganic carbon
  - SWC: soil water content
  - FC: field capacity
- Soil microbial biomass characteristics
  - MBC: microbial biomass carbon
  - MBN: microbial biomass nitrogen
  - MBP: microbial biomass phosphorus
  - MBC:SOC: microbial quotient
- Extracellular enzyme activity characteristics
  - BG: β-1,4-glucosidase
  - BX: β-1,4-xylosidase
  - CBH: Cellobiohydrolase
  - NAG: β-N-acetylglucosaminidase
  - AKP: Alkaline phosphatase
- VL: vector length
- VA: vector angle

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