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Soil moisture mediates microbial carbon and phosphorus metabolism during vegetation succession in a semiarid region

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ABSTRACT

Revegetation of semiarid lands depends upon soil microbial communities to supply nutrients for successive plant species, but microbial activity can be constrained by insufficient water. The objective of this study was to quantify the metabolic limitation of microbes by extracellular enzymatic stoichiometry, and to determine how this affected microbial carbon use efficiency (CUE) with biogeochemical equilibrium model. The study occurred in long-term revegetation experiment with seven successional stages (0, 11, 35, 60, 100, 130 and 150 years) in the Loess Plateau, China. Microbes maintained stoichiometric homeostasis in all successional stages, but plants did not. Microbial metabolism was limited by low soil phosphorus (P) concentration throughout the succession, whereas plants were limited by low soil P during the late successional stages (from 60 to 150 years) only. An increase in soil moisture during succession was associated with greater P limitation in microbes and plants. There was less microbial P limitation at the 35-year successional stage, and the greatest microbial P limitation occurred at the 130-year successional stage. The microbial C limitation followed a unimodal pattern through the vegetation succession and reached a maximum at 100 years of succession (the early forest stage). This coincided with the lowest microbial CUE at 100 years of succession (CUE was from 0.24 to 0.41), suggesting a change in the physiological responses from microbes (such as enzyme synthesis and the priming effect), that tended to reduce soil C sequestration. Our results indicate that soil moisture regulated microbial C and P metabolism during the vegetation succession in this semiarid region, which has implications for understanding how microbial metabolism affects soil C dynamics under vegetation restoration.

1. Introduction

Secondary vegetation succession in disturbed areas is characterized by the changes in plant composition, biomass, soil erodibility, soil nutrient properties, and microbial communities (Finegan, 1984; Cline and Zak, 2016; Wang et al., 2019). It has been receiving increasing attention as an effective natural measure to restore degraded environments (Morriën et al., 2017; Zhong et al., 2018). During this ecological process, plants capture and store a considerable amount of carbon (C) from air into soil, which potentially contribute to mitigating global warming. Moreover, soil microorganisms regulate nutrient cycles through the decomposition and mineralization of soil organic matter (SOM) (Manzoni, 2017; Liang et al., 2017; Cui et al., 2018), which in turn play a key role in plant growth and soil C cycling during secondary succession. Therefore, it is essential to understand the regulation of soil microbial metabolism during vegetation succession in natural ecosystems (Morriën et al., 2017).

Vegetation succession affects soil physicochemical properties by depositing litter and root exudates (Paterson et al., 2007), and consequently causes variation in microbial metabolism. As a result, the

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changes of soil microorganisms affect the availability of soil nutrients, which in turn influence plant growth (Bitas et al., 2013). In particular, vegetation succession also significantly affects soil moisture due to the changes in vegetation types and cover densities as well as soil structure and compaction (Wang et al., 2011; Zhang et al., 2016). The vegetation type and the degree of plant cover can influence evapotranspiration and deep percolation, while soil structure can alter water holding capacities. The variation of soil moisture can directly affect plant growth and microbial activities during vegetation succession, especially in arid and semiarid regions. Furthermore, plant growth is likely limited by a shortage of mineral nutrients such as phosphorus (P) during the establishment of vegetation communities in degraded ecosystems (Zhang et al., 2015; Cui et al., 2018, 2019). Microorganisms decompose organic residues and mineralize SOM as well as compete with plants for nutrients during the periods of nutrient deficiency (Inselsbacher et al., 2010; Cui et al., 2018). Moreover, the soil moisture changes can affect the transportation and availability of soil nutrients (Ouyang et al., 2016), which intensifies or alleviates the competition for nutrients between plants and microbes within microenvironments. Consequently, soil moisture during vegetation succession could strongly couple soil nutrients and microbial metabolism, especially in water-deficient areas. Previous studies related to vegetation succession have almost solely focused on the dynamics of plants, soil nutrients, or microbial communities (Wang et al., 2010, 2019; Morriën et al., 2017; Zhong et al., 2018). However, it is still unclear about the nutrient limitations in microbial metabolism and plant growth, and how these limitations will be varied during vegetation succession in arid and semiarid regions.

Microbial metabolic limitation in soil is attributed to an imbalance in nutrient stoichiometry (Sinsabaugh and Shah, 2012; Cui et al., 2019). Although vegetation succession can aid in the accumulation of C and nitrogen (N) content in soil via photosynthesis and N fixation, P is a relatively stable element in soil (Kirschbaum et al., 2008). The dilution of P content by means of the accumulation C and N in soil might lead to P limitation in microbial metabolism, which in turn potentially increase enzyme activities in P acquisition. SOM decomposition by the exoenzymes from microorganisms is the mediation process of acquiring the nutrients such as N and P (Sinsabaugh et al., 2009; Ge et al., 2017). The C source decomposed from SOM is partly used for the growth of new cells and partly respired for energy production (Manzoni, 2017), which lead to that C allocated to microbial growth may remain within the system whereas C respired is lost (Sinsabaugh et al., 2013). As a result, the consequence of such processes is that microbial metabolic limitation could cause the release of soil C and consequently impact soil C sequestration. Linking microbial metabolic limitation to vegetation succession will consequently reveal the potential mechanisms of soil C pool change in natural ecosystems, especially considering that little is presently known about belowground community metabolic processes that occur under long-term succession. In addition, soil microbial C use efficiency (CUE), defined as the ratio of the rate of microbial growth to the rate of organic matter uptake, is a key parameter in evaluating soil C turnover involved by microorganisms (Manzoni et al., 2012; Geyer et al., 2016). Normally, systems with high CUE effectively retain C within new biomass, whereas low CUE indicates C loss. Hence, it is crucial to identify how microbial CUE relates to metabolic limitation during vegetation succession, which will reveal the response of microbes to ecological processes and understand the dynamics of soil C pool in natural ecosystems.

Therefore, in this study, a chronosequence of secondary succession ages (approximately 150 years) from Ziwuling Forest located in the Loess Plateau, China was selected to investigate the patterns and relationships of microbial metabolic limitation and CUE as well as to explore their drivers during long-term succession in the semiarid region. We hypothesized that: (1) soil microbes and plants more likely to be limited by soil P in the late successional stage compared to the early successional stage; (2) soil moisture could strongly regulate microbial metabolism during the succession in the semiarid region; and (3) microbial CUE will be affected by their metabolic limitation.

2. Materials and methods

2.1. Study area and site selection

The study area was selected in the central of Loess Plateau, located in Ziwuling Mountain region, Gansu Province, China (35°03′-36°37′N, 108°10′-109°08′E), wherein loess hilly topography is typically under the influence of a mid-temperate continental monsoon climate (Zhong et al., 2018). The mean annual temperature and rainfall of the region are 10 °C and 576.7 mm, respectively, with 60% of precipitation occurring from July to August. The soils were derived from primitive or secondary loess parent material (Wang et al., 2010). A deciduous broadleaf forest is the natural biome in the region, which climax community is the *Quercus liaotungensis* Koidz forest (Zou et al., 2002; Wang et al., 2010).

The vegetation community in the Ziwuling Mountain region initially began to be recovered naturally from abandoned cropland due to a national conflict from 1842 to 1866. According to the spatial existence of successional stages, a complete sequence of secondary vegetation stages was progressed in the following order: abandoned farmland, pioneer weeds, herb-shrub mixture, shrubs, early forest, climax forest. The climax forest (*Q. liaotungensis*) was formed after 130–150 years in cropland abandon (Zou et al., 2002; Wang et al., 2010). Correspondingly, we selected six successional stages (11 (R11), 35 (R35), 60 (R60), 100 (R100), 130 (R130), and 150 years (R150)) to evaluate variations in microbial metabolic characteristics during long-term secondary succession. Moreover, an active farmland planted with corn (maize; *Zea mays* L.) was selected as a control (0 years (R0)). To minimize the effect of site conditions on experimental results, all selected sites were similar in elevation, slope gradient, slope aspect, and land use history. Table 1 provided details of each site.

2.2. Soil and plant sampling

Six plots were randomly established at each stage of succession in May 2017, and adjacent plots were separated by 80–100 m. The plots were 20 by 20, 10 by 10, and 2 by 2 m in forest, shrub, and herbaceous communities, respectively (Fig. S1). *Carex lanceolata Boott* is a herbaceous companion species that commonly occurred during all six selected stages of succession. Thus, plant samples from this species were collected during all six successive stages to determine variation in plant chemical elements. These plant samples were oven-dried and ground to measure plant C, N, and P concentration. Soil samples were collected from the top 20 cm soil profile using a stainless-steel corer (5 cm diameter) after removing litter. In total, 10 core samples were collected along an S-shape pattern in each plot and mixed together into one sample. After removing roots, litter, debris, and stones, the collected soil samples were divided into two subsamples. One subsample was immediately placed in an ice box, transported to the laboratory, and then stored at 4 °C for analysis of microbial biomass and enzymatic activity within a period of 2 weeks. The other subsample was passed through a 2 mm sieve and air-dried for physicochemical analysis. Soil bulk density was measured using a soil -bulk sampler and a stainless-steel cutting ring 5 cm in diameter and 5 cm long, at points adjacent to the soil sampling points in each plot.

2.3. Soil physiochemical analysis

Soil moisture content was determined by oven-drying 10 g of fresh soil at 105 °C for 48 h. Soil pH was measured in a 1:2.5 soil:water (w/v) mixture using a glass-electrode meter (InsMark™ IS126, Shanghai, China). Soil organic C (SOC) content was determined using dichromate oxidation. Dissolved organic C (DOC) was extracted with 0.5 M K_2SO_4 and shaken for 60 min at 200 rpm on a reciprocal shaker, and the extracts were measured using a Liqui TOCII analyzer (Elementar,

Table 1
Geographical and vegetation features at different successional stages in the Ziwuling Forest region of the Loess Plateau.

Sites	Successional stage	Latitude (N)	Longitude (E)	Slope (°)	Altitude (m)	Vegetation types	Main species
R0	Farmland	36°4′26″	108°28′6″	10–15	1435	-	-
R11	Pioneer weeds	36°5′2″	108°31′37″	8–12	1335	*B. ischaemun*	*L. dahurica; C. lanceolata*
R35	Herb-shrub mixture	36°5′16″	108°31′35″	10–15	1378	*B. ischaemun* + *L. bicolor*	*H. rhamnoides; C. lanceolata*
R60	Shrubs	36°5′21″	108°31′35″	8–10	1411	*H. rhamnoides*	*S. schneideriana; B. ischaemun; C. lanceolata*
R100	Early forest	36°2′55″	108°31′44″	10–15	1452	*P. davidiana* + *Q. liaotungensis*	*S. schneideriana; C. lanceolate*
R130	Climax community	36°5′22″	108°31′36″	3–7	1479	*Q. liaotungensis*	*S. Salicifolia; O. davidiana Decaisne; C. lanceolata*
R150	Climax community	36°2′55″	108°32′16″	10–15	1477	*Q. liaotungensis*	*S. Salicifolia; O. davidiana Decaisne; C. lanceolata*

Germany) (Jones and Willett, 2006). Total N (TN) content was measured by the Kjeldahl method (Bremner and Mulvaney, 1982). Soil NH_4^+-N and NO_3^--N were analyzed by a continuous flow autoanalyzer after extraction with 2 M KCl with a 1:5 ratio. Total P (TP) and available P (Olsen-P) were extracted with H_2SO_4–$HClO_4$ and sodium bicarbonate (Olsen and Sommers, 1982), respectively, and their contents were then determined by the molybdenum blue method using an ultraviolet spectrophotometer (Hitachi UV2300).

2.4. Measurements of microbial biomass and extracellular enzymatic activity (EEA)

Microbial biomasses C, N, and P (MBC, MBN, and MBP, respectively) were determined by chloroform fumigation-extraction (Brookes et al., 1985; Vance et al., 1987). The experimental procedure has been described in our previous study (Cui et al., 2018). The derived conversion factors were 0.45, 0.54, and 0.40 for MBC, MBN, and MBP, respectively (Joergensen, 1996).

The activities of two extracellular C-acquiring enzymes (β-1,4-glucosidase (BG) and β-D-cellobiosidase (CBH)), two extracellular N-acquiring enzymes (β-1,4-N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP)), and one extracellular organic P-acquiring enzyme (alkaline phosphatase (AP)) were determined using the method of Saiya-Cork et al. (2002) and German et al. (2011). The experimental procedure has also been described in our previous study in detail (Cui et al., 2019). Finally, the enzyme activities were expressed as nanomoles of substrate released per hour per gram of SOM (nmol g SOM^{-1} h^{-1}; SOM = 1.724 × SOC).

2.5. Calculation of stoichiometric homeostasis, microbial nutrient limitation and CUE

Equation (1) was used to calculate the degree of microbial or plant elemental homeostasis (H′).

$$H' = 1/m \quad (1)$$

where m is the slope of $\log_e$ soil (N:P) (resources) versus $\log_e$ microbial (N:P) or slope of $\log_e$ soil (N:P) versus $\log_e$ plant (N:P) scatterplot. $H' \gg 1$ represents strong stoichiometric homeostasis, while $H' \approx 1$ represents weak or no homeostasis (Sterner and Elser, 2002). The regression slope, m, thus was used in this analysis. Data with significant regressions and $0 < m < 1$ were classified as homeostatic ($0 < m < 0.25$), weakly homeostatic ($0.25 < m < 0.5$), weakly plastic ($0.5 < m < 0.75$) or plastic ($m > 0.75$). We classified cases as strictly homeostatic if the regression slope was not significant ($P > 0.05$).

The microbial nutrient limitation was quantified by calculating the vector lengths and angles of enzymatic activity for all data based on untransformed proportional activities (e.g. [BG + CBH]/[BG + CBH + NAG + LAP]). Vector length, representing C limitation, was calculated as the square root of the sum of x^2 and y^2 (Eq. (2)), where x represents the relative activity of C versus P-acquiring enzymes, and y represents the relative activity of C versus N-acquiring enzymes (Moorhead et al., 2016). Vector angle, representing N/P limitation, was calculated as the arctangent of the line extending from the plot origin to point (x, y) (Eq. (3)). Microbial C limitation increases with the vector length. Vector angles >45° represent microbial P limitation, and vector angles <45° represent microbial N limitation. Microbial P limitation increases with the vector angle, and microbial N limitation decreases with it.

$$Length = \sqrt{(x^2 + y^2)} \quad (2)$$

$$Angle\ (°) = \text{DEGREES (ATAN2 } (x, y)) \quad (3)$$

Microbial CUE was calculated using the biogeochemical-equilibrium model (Eqs. (4)–(6)) (Sinsabaugh and Shah, 2012). $EEA_{C:N}$ was calculated as (BG + CBH)/(NAG + LAP), and $EEA_{C:P}$ was calculated as (BG + CBH)/AP. Molar C:X ratios of labile substrate were used as estimates of $L_{C:N}$ and $L_{C:P}$. Labile substrate availability for C, N and P was measured as the quantity of DOC, N and P extracted from non-fumigated control samples in chloroform fumigation analyses. Microbial biomass C:N and C:P ($B_{C:N}$ and $B_{C:P}$) were also calculated as molar ratios:

$$CUE = CUE_{max} \times \{(S_{C:N} \times S_{C:P})/[(K_{C:N} + S_{C:N}) \times (K_{C:P} + S_{C:P})]\}^{0.5} \quad (4)$$

$$S_{C:N} = B_{C:N}/L_{C:N} \times 1/EEA_{C:N} \quad (5)$$

$$S_{C:P} = B_{C:P}/L_{C:P} \times EEA_{C:P} \quad (6)$$

where $K_{C:N}$ and $K_{C:P}$ are half-saturation constants for CUE based on C, N, and P availability. The model postulates that growth rates are maximal when ratios of assimilable nutrient supply match microbial biomass stoichiometry and growth efficiency is proportional to the geometric mean of the N and P supplies relative to C. We assumed that $K_{C:N}$ and $K_{C:P}$ were 0.5 for all model scenarios and that CUE_{max} was 0.6, following Sinsabaugh and Shah (2012).

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine variation in soil physicochemical properties, plant elements, and microbial biomass and metabolic limitation as well as their CUE during secondary vegetation succession, and means were then compared using Tukey's multiple comparisons test ($P < 0.05$) using the R software (v.3.3.2). Pearson correlation was used to examine the relationships among soil physicochemical, plant elemental and microbial characteristics. Linear regression (i.e., the generalized linear model) was adopted to determine the elemental stoichiometric homeostasis in microbes and plants, and the relationships between microbial C and P limitations, and the relationships of the soil P availability with MBP and plant P variations. Nonlinear regression models were adopted to predict variation patterns in microbial metabolic limitation and CUE during vegetation succession.

To identify the relative importance of microbial biomass, plant elements and soil properties in explaining variations in MBP and plant P, variation partitioning analysis (VPA) was conducted from a redundancy analysis (RDA) using the "varpart" function in the "Vegan" package in R. Furthermore, we determined the relative influence of each variable using linear models in the "relaimpo" package (Grömping, 2006). To explore cascading relationships between microbial metabolic limitation and CUE with environmental variables, all significant environmental variables were divided into three categories (succession time, soil

properties and microbial biomass). Partial least squares path modelling (PLS-PM) was used to further identify possible pathways whereby variables control microbial metabolic limitation and CUE during vegetation succession. The models were constructed using the "innerplot" function in the "plspm" package. All statistical analyses were performed using the R software (v.3.3.2).

3. Results

3.1. Vector characteristics of extracellular enzymatic stoichiometry

Characteristics of enzymatic stoichiometry differed among the successional stages (Fig. 1 A). All data points were above the line (the 1:1 line), indicating strong P limitation in the microbial community during the succession. The relative microbial C limitation had a wider range compared to P limitation because the clusters exhibited broader distribution along the diagonal. Microbial C and P limitations were quantified by calculating vector lengths and angles using relative proportional enzymatic activities (Fig. 1B and C). Vector lengths and angles (ranging from 0.457 to 0.706 and from 59.3° to 64.5°, respectively) changed significantly during the succession ($P < 0.001$). For example, the vector length and angle (0.457 ± 0.06 and 59.3° ± 1.66°, respectively) were the lowest at R11 and R35, respectively, suggesting that the lowest microbial C and P limitations occurred in the pioneer weeds (R11) and herb-shrub mixture (R35) stages. Linear regression analysis showed a significantly negative correlation between vector length and angle at the successional stage of 0–60 years, while a significantly positive correlation at the successional stage of 60–150 years ($P < 0.01$, Fig. 1 D).

3.2. Stoichiometric homeostasis in microorganisms and plant species

In order to test the strength of stoichiometric homeostasis in microorganisms and plant species, we analyzed relationships between elemental ratios of microbial biomass or plant species and soil nutrient ratios. For microorganisms, when the combined data were analyzed, no significant correlations were found between microbial N:P and soil N:P ($P > 0.05$), which indicated strong community-level elemental homeostasis during the succession (Fig. 2 A). For plants, however, there was significantly positive correlation between plant N:P and soil N:P ($y = 1.07x + 0.05$; $P < 0.001$), and the regression slope was close to 1 (Fig. 2 B). This indicated that there was no elemental homeostasis in plant species during the succession.

Furthermore, no significant correlation was found between plant P and Olsen-P at the successional stage of 0–60 years, while a significantly negative correlation between plant P and Olsen-P was observed at the successional stage of 60–150 years ($y = -0.12x + 2.05$; $P < 0.001$, Fig. 3). The clear separation of scatters points for the two successional stages also indicated that there were different relationships between plant P and Olsen-P at the succession stages of 0–60 years and 60–150 years. In addition, plant P was significantly negatively correlated to soil moisture, and plant elemental ratios (C:P and N:P) were positively correlated to soil moisture ($P < 0.001$, Fig. S6). However, positive correlations between microbial biomass (MBC, MBN and MBP) and soil moisture were observed ($P < 0.001$; Fig. S7). There were no significant

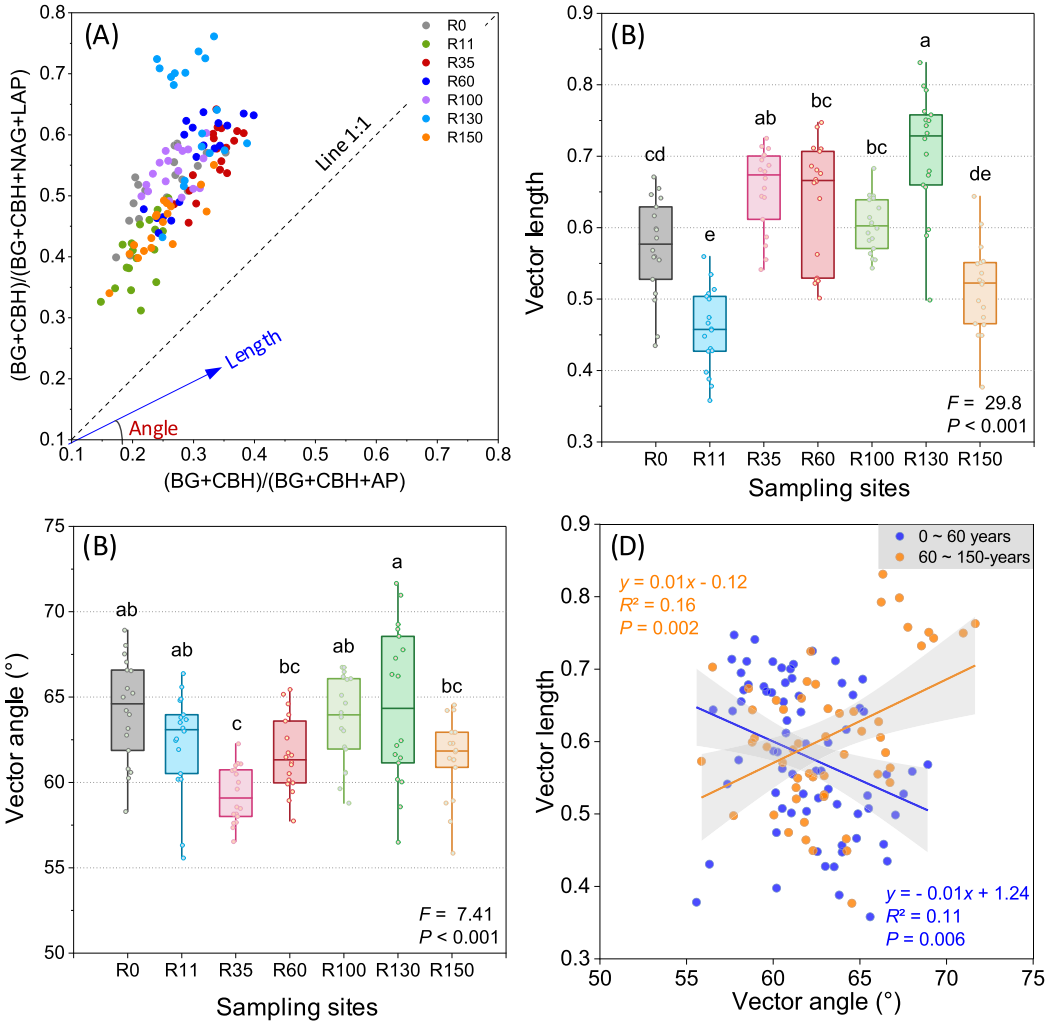

Fig. 1. Enzymatic stoichiometry of the relative proportions of C to N acquisition versus C to P acquisition (A), the variation of vector length and angle (B and C) and their relationships (D). (A): BG, β-1,4-glucosidase; CBH, β-D-cellobiosidase; NAG, β-1,4-N-acetylglucosaminidase; LAP, L-leucine aminopeptidase; AP, alkaline phosphatase; vector length represents soil C limitation for microbes, vector angle represents soil N/P limitation for microbes. (B and C): Values are the means ± standard error ($n = 18$). Different letters indicate significant differences ($P < 0.05$) among the successional stages based on one-way ANOVA followed by Tukey's test. (D): Linear-regression analysis to identify the relationships of microbial C limitation with microbial N/P limitation during the early and late successional stages.

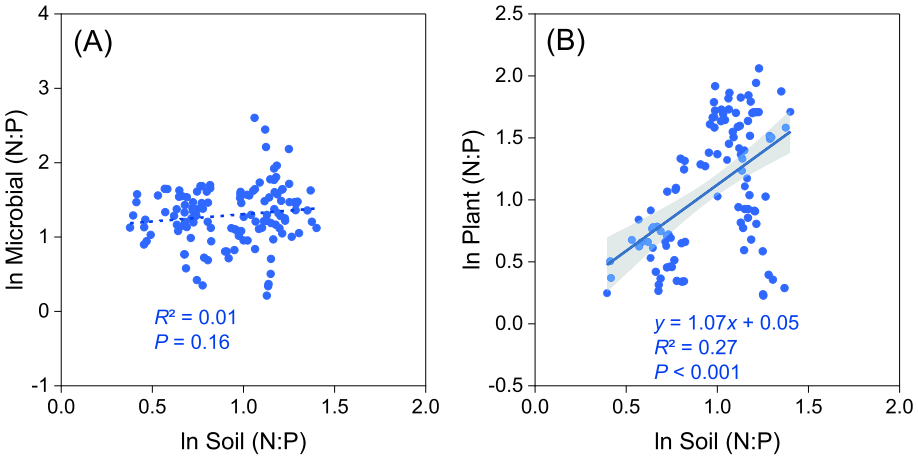

Fig. 2. The identification of elemental homeostasis for soil microorganisms (A) and plants (B). (A): The relationships of microbial N:P ratio with soil N:P ratio. (B): The relationships of plant N:P ratio with soil N:P ratio. The data is logarithmically transformed. The regression slope $\ll 1$ and is not significant ($P > 0.05$) that represents strong stoichiometric homeostasis, while slope ≈ 1 and is significant ($P < 0.05$) that represents weak or no homeostasis. Blue solid line indicates the model fits between the plant (N:P) and soil (N:P), and grey areas are the 95% confidence intervals of the model. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

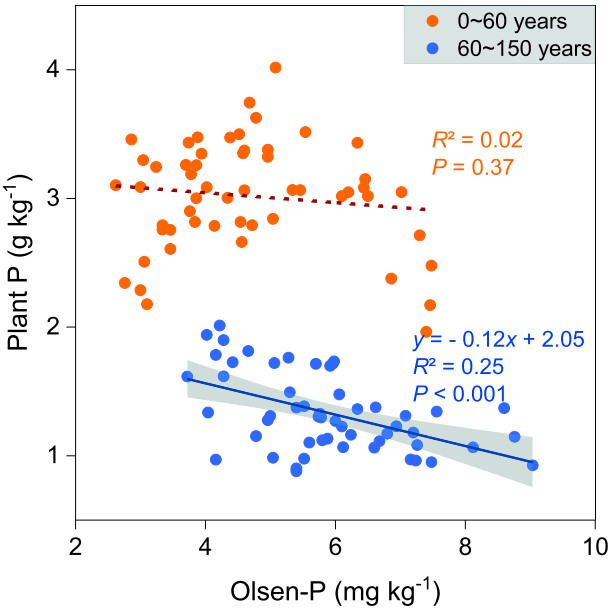

Fig. 3. Relationships between plant P and available soil P during the early and late successional stages. Early successional stage, 0–60 years; late successional stage, 60–150 years. Blue solid line indicates the model fits between the plant P and Olsen-P, and grey areas are the 95% confidence intervals of the model. Plant P, plant P concentration; Olsen-P, soil available P content. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

correlations between microbial biomass ratios (MBC:MBN, MBC:MBP and MBN:MBP) and soil moisture.

3.3. Variations in soil properties, plant elements and microbial biomass, as well as their effects on MBP and plant P

Vegetation succession caused significant variation in soil nutrients ($P < 0.05$, Fig. S2). SOC and TN contents increased significantly after 60 years succession and had the maximum values at R150 (22.2 ± 1.19 and 2.02 ± 0.17 g kg^{-1}, respectively). There was a significant decrease in soil pH over time wherein the minimum (8.06 ± 0.12) occurred at R100. Notably, soil moisture significantly increased after 60 years succession and peaked (21.7 ± 2.78%) at R130. This increasing tendency during the late successional stage was confirmed at different sampling times (Table S1). Nutrient concentrations of *Carex lanceolate Boott* also changed significantly during the succession ($P < 0.05$, Fig. S3). Particularly, P concentration significantly decreased after 60 years succession with the minimum value at R130 (1.16 ± 0.15 g kg^{-1}). Correlation analysis showed that the most soil physicochemical properties were significantly correlated with plant elements and their stoichiometry during the successional stage of 60–150 years ($P < 0.05$, Fig. S5). For the microbial biomass, MBC, MBN and MBP significantly increased after 60 years succession ($P < 0.05$, Fig. S4). And most soil physicochemical properties were significantly correlated with microbial biomass and their stoichiometry during the succession ($P < 0.05$, Fig. S5). It is interesting to note that, compared to the successional stage of 0–60 years, stronger correlations were observed between plant element stoichiometry and microbial properties (microbial biomass and their stoichiometry) at the successional stage of 60–150 years.

VPA showed that microbial biomass, plant elements and soil properties explained 30%, 20% and 45% of MBP variation, respectively, at the successional stage of 0–60 years, while they explained 2.0%, 1.0% and 63% of MBP variation, respectively, at the successional stage of 60–150 years (Fig. 4 A and C). Microbial biomass, plant elements and soil properties explained 18%, 1.0% and 18% of variation in plant P, respectively, at the successional stage of 0–60 years, while they explained 27%, 3.0% and 63% of variation in plant P, respectively, at the successional stage of 60–150 years (Fig. 4 B and D).

In the soil, microbial biomass, plant elements and soil properties typically explained 72.11% of MBP variation at the successional stage of 0–60 years. Soil moisture and SOC were the most important drivers (with a 8.76% and 7.51% relative influence, respectively; Fig. 5 A). Similarly, these variables explained 67.86% of MBP variation at the successional stage of 60–150 years, while Olsen-P was the most important driver (with a 14.01% relative influence; Fig. 5 C). In terms of plant, microbial biomass, plant elements and soil properties typically explained 33.16% of plant P variation at the successional stage of 0–60 years, and soil moisture was the most important driver (with a 6.37% relative influence; Fig. 5 B). However, these variables explained 75.04% of plant P variation at the successional stage of 60–150 years, and pH and Olsen-P were the most important drivers (with a 17.88% and 13.31% relative influence, respectively; Fig. 5 D).

3.4. Microbial CUE and its drivers during the succession

Microbial CUE varied significantly among the different successional stages with a mean value of 0.300 ($P < 0.001$, Fig. 6). The highest microbial CUE was 0.409 ± 0.05 at R11, whereas the lowest one was 0.236 ± 0.05 at R130. The PLS-PM indicated that both changes of soil moisture and nutrient stoichiometry induced by the vegetation succession affected soil nutrient availability and microbial biomass, which ultimately affected microbial metabolic characteristics (Fig. 7A and B). At the successional stage of 0–60 years, the total effects of MBP (−0.176),

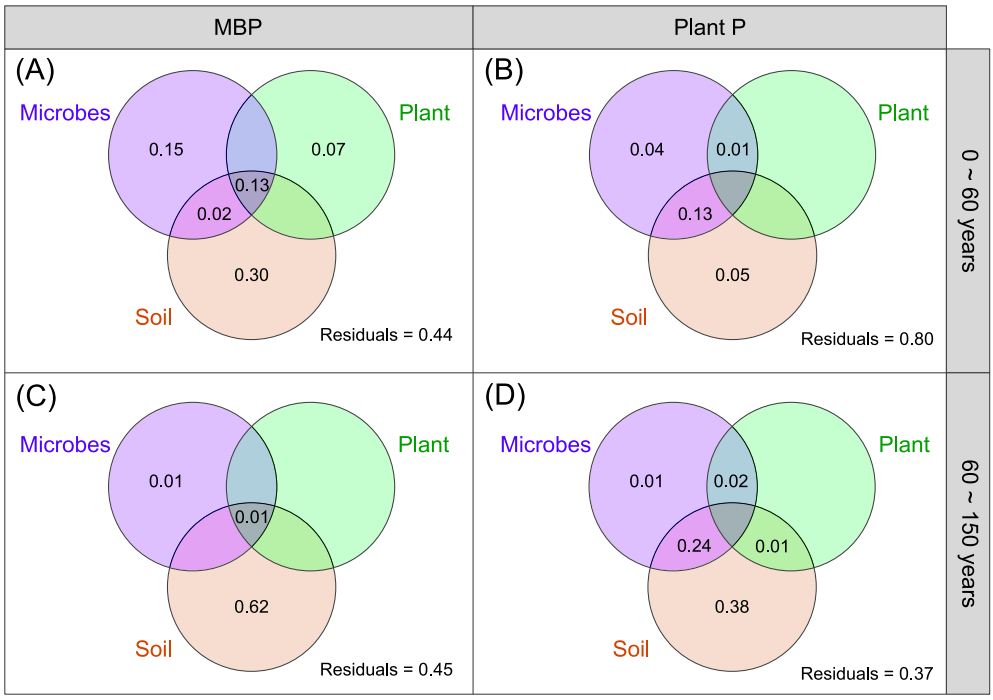

Fig. 4. Variation partitioning analysis (VPA) showing the effects of microbes, plants and soil on MBP (A and C) and plant P (B and D) during the early and late successional stages. Early successional stage, 0–60 years; late successional stage, 60–150 years. Microbial biomass is MBC and MBN, and plant element concentration is plant C, plant N and plant P in the (A) and (C). Microbial biomass is MBC, MBN and MBP, and plant element concentration is plant C and plant N in the (B) and (D). Soil properties include SOC, soil organic C; TN, soil total N; TP, soil total P; DOC, soil dissolved organic C; TAN, NO_3^--N + NH_4^+-N; Olsen-P, soil available P; SOC:TN, the ratio of SOC to TN; SOC:TP, the ratio of SOC to TP; TN:TP, the ratio of TN to TP; pH; soil moisture content; BD, bulk density.

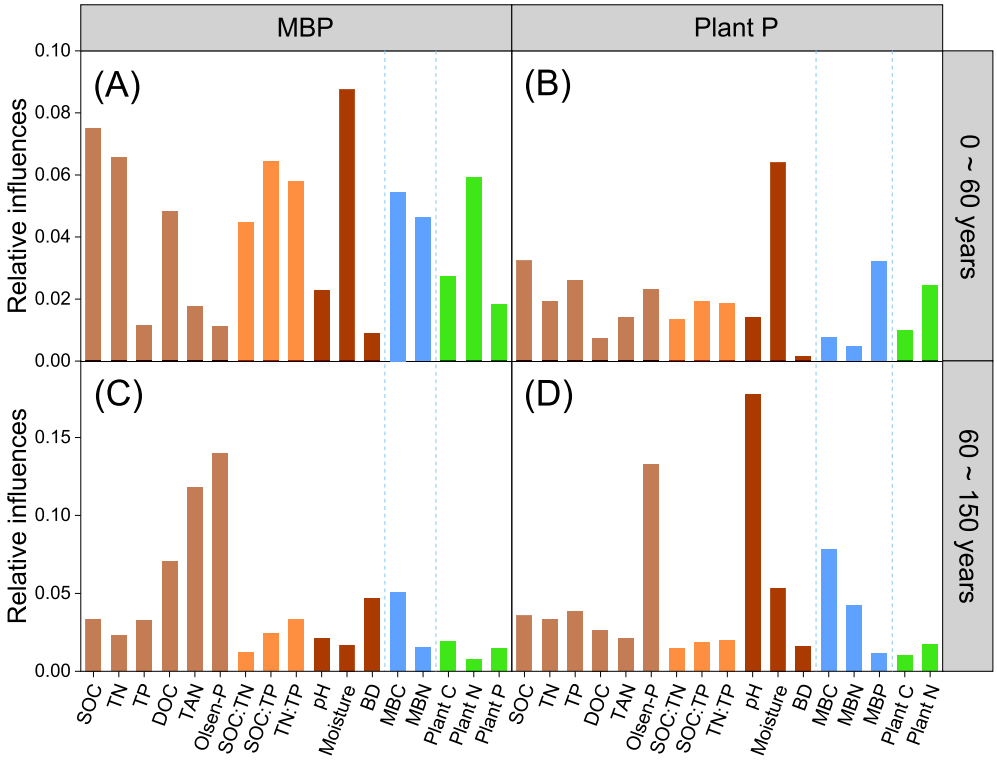

Fig. 5. Relative influences of environmental drivers (microbes, plants and soil) on MBP (A and C) and plant P (B and D) during the early and late successional stages. Early successional stage, 0–60 years; late successional stage, 60–150 years. Soil properties include SOC, soil organic C; TN, soil total N; TP, soil total P; DOC, soil dissolved organic C; TAN, NO_3^--N + NH_4^+-N; Olsen-P, soil available P; SOC:TN, the ratio of SOC to TN; SOC:TP, the ratio of SOC to TP; TN:TP, the ratio of TN to TP; pH; soil moisture content; BD, bulk density. Microbes include MBC, MBN and MBP. Plant include plant C, plant N and plant P.

MBC and MBN (−0.128) and MBC and MBN (0.365) were the highest for microbial P limitation, microbial C limitation and CUE, respectively (Fig. 7 C). At the successional stage of 60–150 years, however, the total effects of Olsen-P (0.460), DOC and TAN (0.449) and DOC and TAN (−0.380) were the highest for microbial P limitation, microbial C limitation and CUE, respectively (Fig. 7 D). Furthermore, microbial C limitation led to microbial P limitation at the successional stage 0–60 years, while microbial P limitation led to microbial C limitation at the successional stage of 60–150 years. Additionally, microbial C limitation was the direct driver of microbial CUE variations throughout the entire succession.

3.5. Variation patterns of microbial metabolic limitation and CUE

Nonlinear regression analysis showed that microbial C limitation and CUE along vegetation succession time fitted to the quadratic function

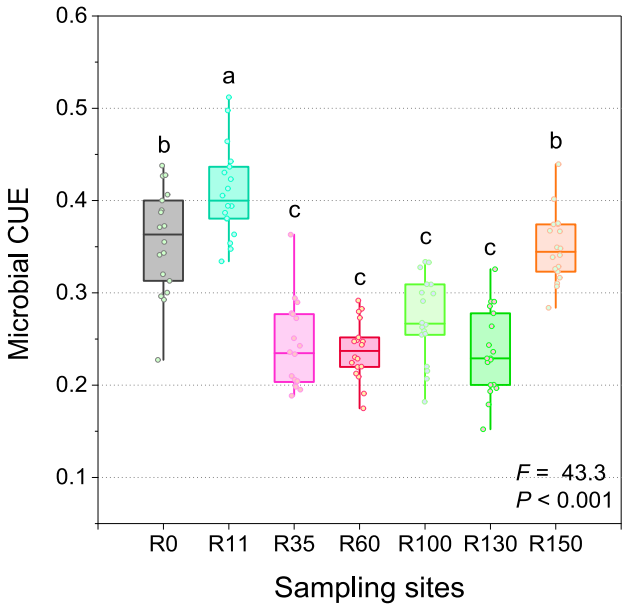

Fig. 6. Box plots showing microbial carbon use efficiency (CUE) among different successional stages. Values are the means ± standard error ($n = 18$). Different letters indicate significant differences ($P < 0.05$) among the successional stages based on one-way ANOVA followed by Tukey's test.

models, and microbial P limitation fitted to the cubic function model ($P < 0.001$, Fig. 8). The quadratic function models ($y = -3 \times 10^{-5}x^2 + 0.006x + 0.428$, $R^2 = 0.353$; $y = 3 \times 10^{-5}x^2$ -0.005x + 0.433, $R^2 = 0.509$) estimated the maximum microbial C limitation (0.728) and the minimum microbial CUE (0.225) at 100 and 83 years succession, respectively (Fig. 8 A and C). The cubic function model ($y = -2 \times 10^{-5}x^3 + 0.005x^2$ - 0.268x + 64.6, $R^2 = 0.243$) estimated that microbial P limitation had the maximum and minimum values (70.33 and 60.46, respectively) at 133 and 34 years successions, respectively (Fig. 8 B).

4. Discussion

4.1. Soil moisture mediation of plant P limitation during long-term secondary succession

Microbial communities maintained strong stoichiometric homeostasis during long-term secondary succession in this study (Fig. 2 A), which was supported by the results from Li et al. (2019). Soil moisture had no significant effect on microbial biomass stoichiometry, which confirmed the existence of stoichiometric homeostasis in microbial communities during the succession (Fig. S7). One of the reasons could be that microorganisms can obtain relatively scarce resources by optimizing the allocation of C, N, and P during enzymatic synthesis processes (Sinsabaugh et al., 2009; Cui et al., 2018).

To the opposite with the microbial community, no stoichiometric homeostasis was observed in the specific plant species (*Carex lanceolate Boott*) during the succession (Fig. 2 B). Previous studies also reported that C:N:P stoichiometry patterns exhibit significant flexibility in plants in the arid and semiarid regions (Ren et al., 2016; Zeng et al., 2017). Different from the significantly varied plant P concentration with soil Olsen-P during the late successional stage (60–150 years), however, the plant P concentration did not significantly change with it during the early successional stage (0–60 years) (Fig. 3). Moreover, the P concentrations in plant were also significantly higher at the early successional stage than that at the late successional stage (Fig. S3). These results suggested that plants could be limited by soil P at the late successional stage (the forest stage), but not at the early successional stage, which seems to support our first hypothesis. This was further confirmed by VPA analysis because soil properties and microbial biomass had relatively small effects on plant P during the early successional stage (Fig. 4 B), while they had a strong effect on plant P during the late successional stage (Fig. 4 D). This suggested an increase in soil P limitation and P competition between microbes and plants during the forest successional stage. Dilution effects in soil P caused by soil C and N accumulation during vegetation succession also could result in or aggravate P limitation in plants during the late successional stage (Groenigen et al., 2006).

It is interesting to observe the significantly negative relationship between plant P concentration and soil Olsen-P content at the late successional stage (Fig. 3). Soil moisture was the most important factor affecting plant P concentration during the early successional stage, while pH and Olsen-P were the most important factors affecting it during the late successional stage (Fig. 5 B and D). Decreased soil pH can alter complexed P and will make P more available to microbes and plants in the alkaline soils. These suggested that plant growth was likely mainly limited by soil moisture during the early successional stage and limited by soil P availability during the late successional stage. Soil water is the main factor to vegetation growth in arid and semiarid ecosystems (Wang et al., 2011). The significantly higher soil moisture during the forest stage compared to the herb-shrub stage could support more plant growth, which thus result in greater aboveground community biomass. Consequently, as soil moisture dramatically increased during the late successional stage, a negative relationship occurred between plant P concentration and soil Olsen-P content in P deficient soils. It further confirmed that plants could be limited by soil P at the forest successional stage, and soil moisture was the key driver of plant P limitation during this stage.

However, we selected only one plant species (*Carex lanceolata Boott*) that commonly occurred during all six selected stages in the present study. The results could be insufficient to reflect the nutrient limitationof the entire plant community. Perhaps it is necessary to sample all the dominant species at each stage to compare the common species at all stages. In this case, however, we likely can't exclude the effects of physiological differences between species. Anyway, our current results take a new step and are valuable for understanding vegetation community dynamics during the succession.

4.2. The relationship between microbial C and P limitations during vegetation succession

The extracellular enzymatic stoichiometry model revealed that microbial metabolism underwent relative C and P limitations in soil throughout the entire succession (Fig. 1B and C), which partly confirmed our first hypothesis. Furthermore, the significant correlations between microbial C and P limitations suggested a strong dependence between them during the succession (Fig. 1 D), which was also found in our previous study (Cui et al., 2019). It is interesting to note their opposite relationships during the early and late of successions (Fig. 1 D). This suggested that their relationships could strongly depend on the levels of C or P limitation. This microbial P limitation could either occur or increase because P consumption by root systems could reduce soil P availability and impede P acquisition by microorganisms (Inselsbacher et al., 2010). A strong P limitation in plants during the late successional stage implied that plants and microbes inevitably compete for soil P during this stage (Fig. 3; Cui et al., 2018). The P requirement of microbes could motivate their metabolism, resulting in a relative microbial C limitation at the late successional stage. Previous study reported that if P becomes relatively restricted, microbial communities may expend more C and N to produce and excrete enzymes associated with P metabolism (Marklein and Houlton, 2012). Furthermore, most P required by microbes do derives from SOM decomposition (Tarafdar and Claassen, 1988; Ru et al., 2018). Therefore, the high P limitation could enhance C metabolism (high relative C limitation) in microbes during the late successional stage.

In terms of MBP, specifically, soil moisture and SOC were the most

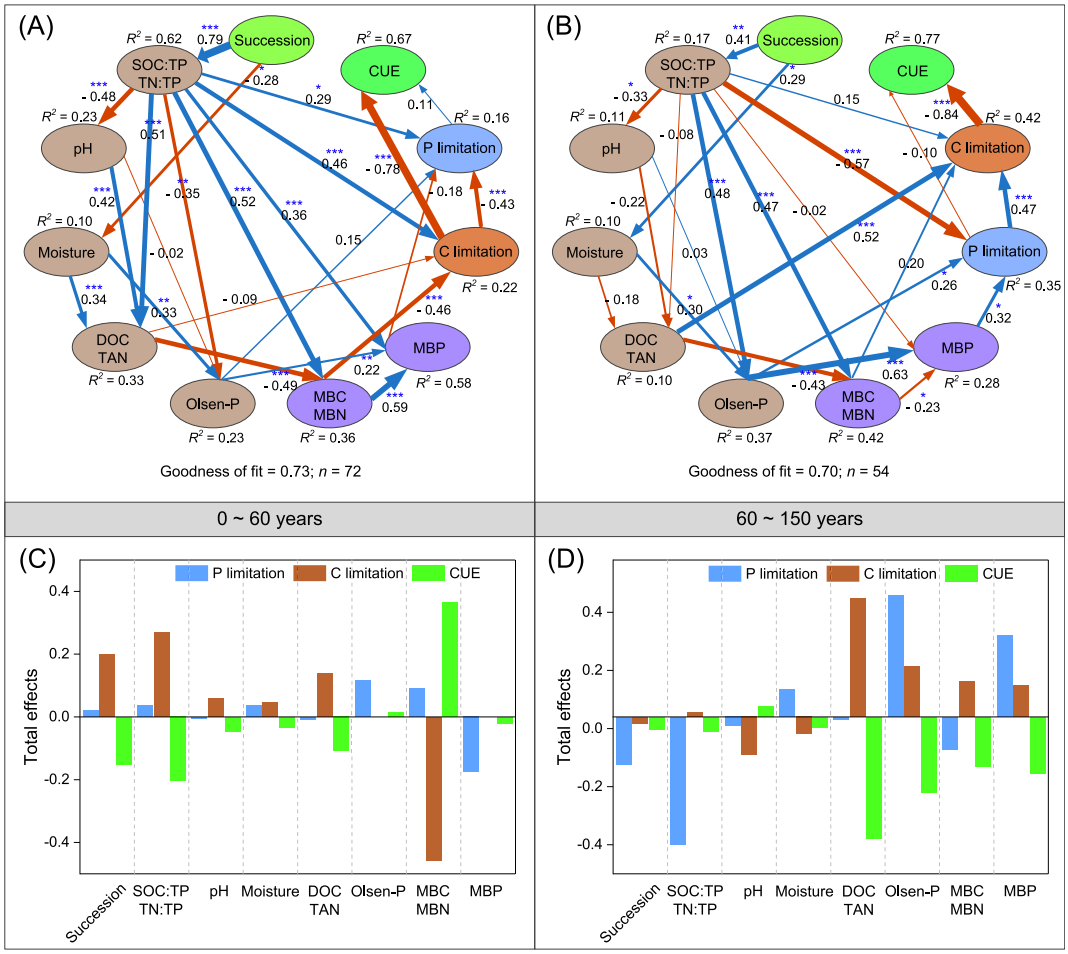

Fig. 7. Cascading relationships of microbial nutrient limitation and carbon use efficiency (CUE) with succession times, soil properties and microbial biomass. Partial least squares path modelling (PLS-PM) disentangling major pathways of the influences of succession times, soil physicochemical properties and microbial biomass on microbial C limitation (represented by vector length), microbial P limitation (represented by vector angle), and microbial CUE during the early (A and C) and late (B and D) successional stages. Early successional stage, 0–60 years; late successional stage, 60–150 years. Blue and yellow arrows indicate positive and negative flows of causality ($P < 0.05$), respectively. Numbers on the arrow indicate significant standardized path coefficients. R^2 indicates the variance of dependent variable explained by the model. Soil properties include SOC:TP, the ratio of SOC to TP; TN:TP, the ratio of TN to TP; DOC, soil dissolved organic C; TAN, NO_3^--N + NH_4^+-N; Olsen-P, soil available P; pH; soil moisture content. Microbial biomass includes MBC, MBN and MBP. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

important factors affecting MBP during the early successional stage, while Olsen-P was the most important factor affecting MBP during the late successional stage (Fig. 5 A and C). This suggested that the microbial community could be more limited by soil moisture and soil C during the early successional stage while more limited by soil P during the late successional stage, which partly confirmed our second hypothesis. This was also supported by the relative low soil moisture and SOC content at the early successional stage (Fig. S2). Generally, microbial metabolism in soil is inhibited under low soil water conditions, implying a reduction in microbiological activity (Borken et al., 2006). Thus, soil water availability constrains litter decomposition and nutrient release through its effects on decomposer community activity. The PLS-PM also indicated that soil moisture directly affected soil nutrient availability, and indirectly affected microbial C limitation (Fig. 7A and B). Thus, relatively low soil moisture and C availability could lead to a strong microbial C limitation during the early successional stage. Strong C limitation represents a high potential for SOM decomposition due to high C-acquiring enzyme activities. The decomposition of SOM could further release P, which then alleviate microbial P limitation. Consequently, microbial P limitation showed a negative relationship with relative C limitation during the early successional stage.

A deficiency in soil P is unable to supply a sufficient amount of inorganic P for the synthesis of phosphate compounds (e.g., adenosine triphosphate (ATP), DNA, and RNA), and this may contribute in restricting plant nutrient uptake and microbial metabolism (Elser et al., 2003). Our results suggested that the plant (*Q. liaotungensis*) community could have been exposed to the strongest P limitation at 130 years succession (Fig. 1 C). The P limitation of plants and microbes could be alleviated by the return of plant residues after 130 years succession because of the significantly decreased P limitation at 150 years succession. Cherif and Loreau (2007) also reported that resource availability is likely a fundamental driver of both microbial and plant succession. Soil P limitation in microbes and plants could strongly affect belowground ecological functions and the stability of the vegetation community during its development; hence, this could also be an important reason leading to vegetation degeneration in the semiarid regions (Wang et al., 2011; Liu and Shao, 2015; Cui et al., 2019). Furthermore, the fitting model predicted that P limitation decreased after 133 years succession (Fig. 8). As succession progressed, P limitation in both plants and microbes were continuously decrease. Therefore, we inferred that it would take at least 130 years for forest succession to reach a stable stage in the semiarid regions of the Loess Plateau.

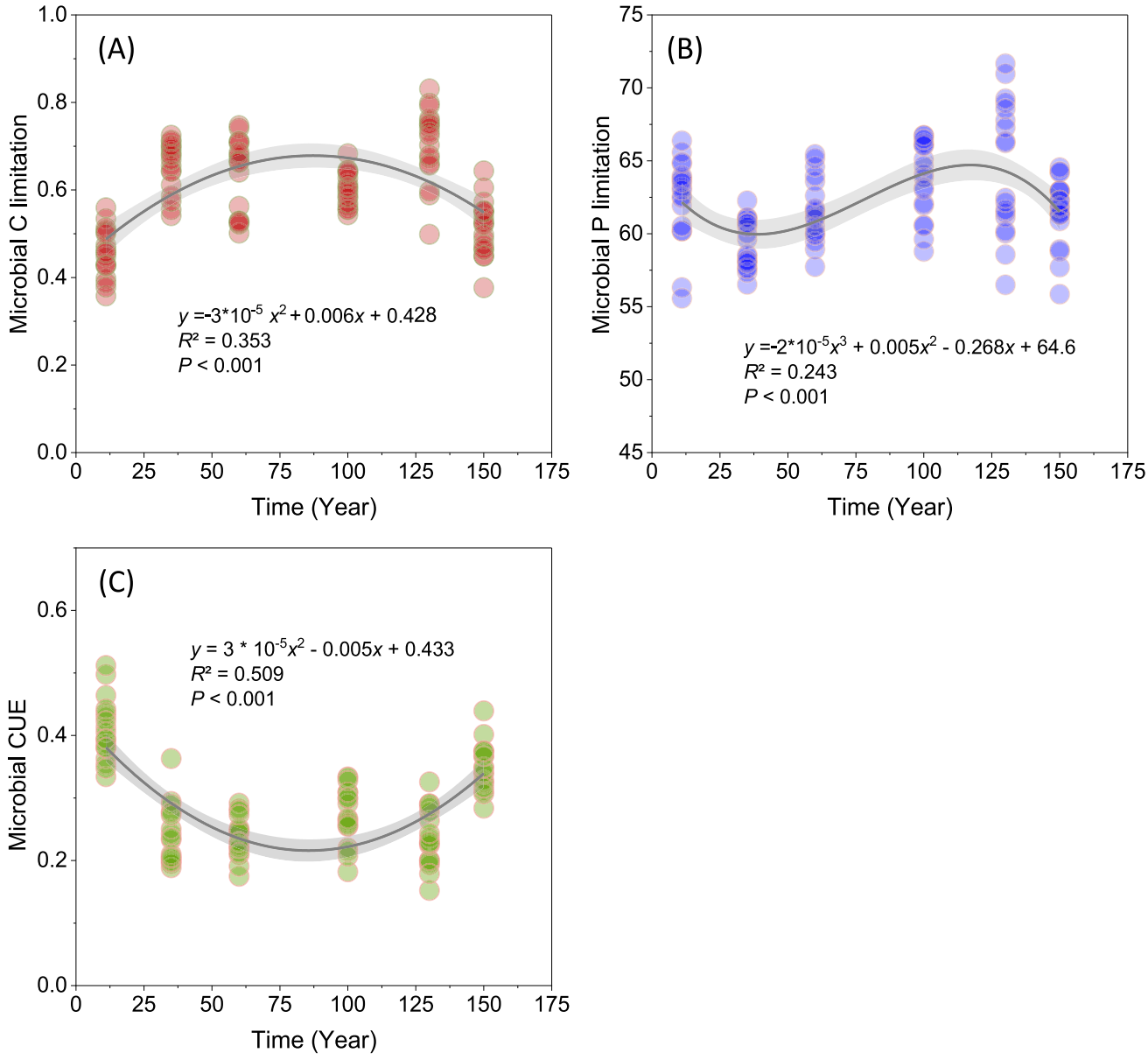

Fig. 8. Variation patterns of microbial nutrient limitation and carbon use efficiency (CUE) during vegetation succession. (A): microbial C limitation is represented by vector length, (B): microbial P limitation is represented by vector angle. Black lines indicate the models fit between microbial nutrient limitation/CUE and successional time, while grey areas are the 95% confidence intervals of these models.

4.3. Implications of microbial C and P limitations for soil C turnover during long-term succession

In the present study, the microbial CUE was quantified as a mean of 0.300 during the succession (Fig. 6), which was similar with the results from other studies (Sinsabaugh et al., 2013; Geyer et al., 2019). After linking microbial metabolic limitation to their CUE, the microbial CUE pattern was roughly opposite to that of microbial C limitation during the succession (Fig. 8). PLS-PM and correlation analysis further confirmed that microbial metabolic limitation had a strong negative effect on microbial CUE (Figs. 7 and S5), which supported the third hypothesis of this study. As microbial metabolic limitation increase, the corresponding decrease in CUE could be attributed to a shift from growth to maintenance respiration and an increased investment in the production of enzymes as decomposition progresses (Manzoni et al., 2012). Specially, microbial metabolic limitation, being the most important metabolic characteristic, could affect CUE in two ways. On the one hand, the availability of soil nutrients is directly involved in the growth and metabolism of microorganisms. When available nutrients are deficient, such as the C and P limitations in our study area, microorganisms must first break down complex compounds (Agren and Bosatta, 1987). Compounds that require many enzymatic steps for degradation may decrease conversion efficiency into new biomass because the production of heterotrophic microbial communities is used to make enzymes for secretion into the environment (Sinsabaugh and Shah, 2012). Additionally, various substrates require different metabolic pathways to complete assimilation by microorganisms, which may lead to a wide range of respiration rates per unit C assimilated (Pawvan et al., 2005). Consequently, these processes would greatly decrease microbial CUE (Fig. 9).

On the other hand, during vegetation succession, the priming effect could be another significant mechanism to regulate CUE. Priming effect is strong short-term changes in the turnover of SOM caused by pulses or continuous inputs of fresh organics (e.g., plant litter and root exudates)

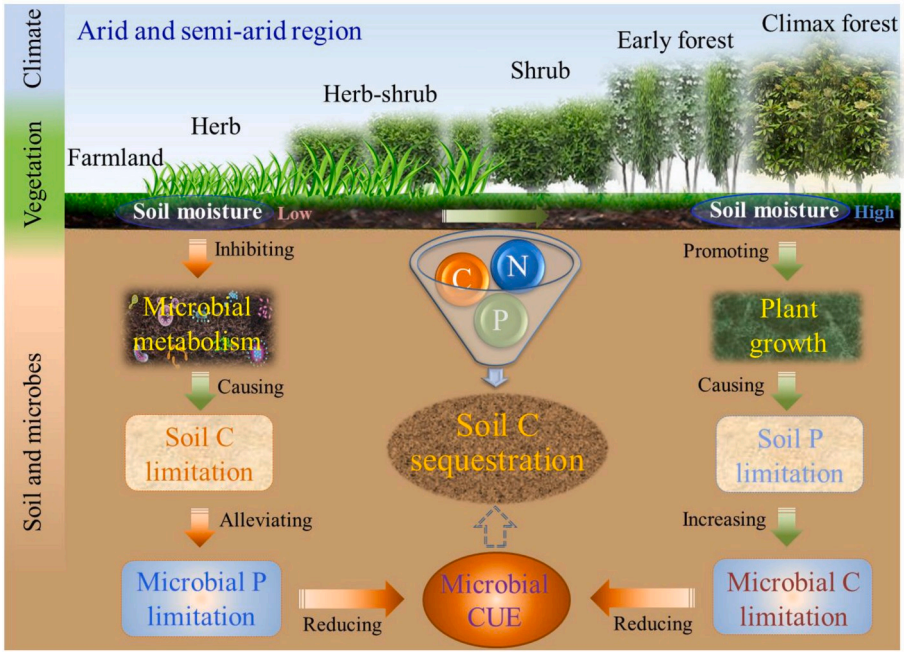

Fig. 9. Conceptual diagram for the influence of soil moisture change on processes controlling soil microbial metabolism during the secondary succession.

(Kuzyakov et al., 2000). This process can lead to that large amounts of C and other nutrients are released as "primed" CO_2 or immobilized in soil due to increased activity or amount of microbial biomass (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010). Furthermore, priming frequently occurs or strengthens when microorganisms become nutrient-limited during the degradation process of new labile C and co-metabolize preexisting SOM to meet their nutrient demands (Nottingham et al., 2012; Luo et al., 2017). For example, priming effects resulting from the co-metabolism of organic matter by nutrient-limited microorganisms have also been identified by an increase in microbial synthesis of extracellular enzymes, which liberates nutrients from organic matter (Nottingham et al., 2012; Yu et al., 2018). As a result, compared to microbial C assimilation, a higher proportion of C could be released to atmosphere due to soil priming effect induced by both microbial metabolic limitation and plant C input during the succession (Fig. 9). Considering long-term secondary succession can accumulate abundant organic matter, the priming effect from nutrient limitation would thus have considerable consequences for forest C sinks by destabilizing SOC and reducing microbial CUE (Fig. 9). The SOM decomposition by microbes with low CUE could inevitably offset part of plant C fixation, and will lead to a positive feedback to current global warming.

5. Conclusions

Revegetation in semiarid regions is expected to increase soil C sequestration. This depends on the ability of the soil microbes to maintain metabolic functions related to decomposition and nutrient cycling. Since microbes maintain stoichiometric homeostasis during succession, whereas plants do not, this requires microorganisms to adjust their CUE and consequently affects the soil C sequestration. Furthermore, a lack of soil moisture will limit microbial metabolism during succession, meaning that the periods when microbes are most C- and nutrient-limited will have lower soil C sequestration than successional stages without these limitations. Additional study is required to distinguish the mechanisms responsible for nutrient limitation of microbes and plants during vegetation succession. We also recommend to quantify the contribution of microbial metabolic limitation to soil C dynamics with other approaches, such as stable isotope labelling and microcosm experiments.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107814.

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