Land use type dominates soil microbial element limitations in a subtropical plateau, China

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Abstract. Land use type, vegetation type (artificial or natural) and microbes play a key role in flux of soil matter and energy in subtropical ecosystems. However, microbial element limitations in these ecosystems have been largely unexplored. Here, microbial element limitations under four representative land uses grouped into two vegetation types (artificial or natural) were characterized using enzymatic vector analysis, their drivers were deciphered using redundancy analysis in a subtropical region, Southwestern China. Enzymatic vector analysis showed that soil microbes suffered from carbon (C) & nitrogen (N) limitations in cropland and forestland related to artificial vegetation, but C, N & phosphorus (P) limitations in grassland and shrub land related to natural vegetation. Two-way ANOVA showed land use type and vegetation type (artificial/natural) were the major forces driving C and NP limitations. Redundancy analysis identified that soil total N was the major factor affecting soil microbial C limitation, soil total P was the major factor affecting soil microbial NP limitations. This study implied that conversion of natural vegetation to artificial vegetation would shift microbial element limitations from C, N & P limitation to C & N limitation, and exacerbate N limitation.

Keywords: Land use type; soil enzyme activities; driving forces; microbial element limitations; subtropical region.

1. Introduction

Soil microbes play an important role in material and energy cycle (Camenzind et al. 2018), plant productivity (Ding et al. 2020a) and soil carbon fixation and organic matter degradation (Cui et al. 2019a), which have received growing attention recently (Chen et al. 2019a). Understanding resource limitation to soil microbes is therefore, crucial for modeling ecosystem functions (Chen et al. 2019a). However, our exploration of resource limitation for soil microbes has just begun. Soil microbes acquire carbon and nutrients mainly by producing extracellular enzymes to depolymerize resource, e.g. organic matter (Cui et al. 2019a, Ding et al. 2020a). Land use change has been documented to change soil resource for microbial growth (Chen et al. 2019a), we thus expect that land use type can change soil microbial resource limitations. The objectives of this study were thus to characterize microbial element limitations under the representative land uses and decipher their drivers.
Material and method

2.1. Study site
The study was conducted in Longli county in the Guizhou Plateau, Southwestern China (26°19′-26°23′N, 106°50′-106°54′E, 1450 m above sea level). The climate is a subtropical monsoon climate with annual rainfall of 1160 mm and an average temperature of 14.8 °C, ranging from -3°C in January to 35°C in July (Ding et al. 2019). This area is characterized by Haplic Alisols (Ding et al. 2020b).

2.2. Soil sampling
In October 2017, soil samples were collected from two land uses related to artificial vegetation [i.e., cropland (maize land, CL) and afforested land (secondary forestland, FL)] and two land uses related to natural vegetation [i.e., shrub land (natural shrub land, SL) and grassland (natural grassland, GL)] with three plots as three independent replicate plots (30 m × 30 m). The distance between each plot at least 10 m. Analogous to many studies, to minimize differences from non-deigned sources (Ding et al. 2020b), the three plots at same land use was similar in plant community composition, structure and population density. Due to the shallow soil depth (Ding et al. 2019), we selected only 0–30 cm soils with intervals of 0-10, 10-20 and 20-30 cm using a soil auger (Ding et al. 2019). One subsample was stored at 4°C until analyses for soil enzyme activity. The other was air dried for SOC (soil organic carbon), STN (soil total nitrogen), and STP (soil total phosphorus) analyses.

2.3. Soil physicochemical assay
Soil moisture (SWC) and soil bulk density (BD) were obtained using the cutting ring method (Ding et al. 2019). The assay method of soil pH, SOC, STN and STP were listed by Ding et al. (2020a).

2.4. Soil enzyme activities assay
The activities of β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase, leucine aminopeptidase and acid phosphatase were measured following the fluorometric techniques (Ding et al. 2020a). Microbial element limitations were calculated using the function (https://github.com/dlltargeting/evcmdl) (Ding et al. 2020a) based on the vector analysis of the log-transformed ratio enzyme activity (vector length and vector angle) (Moorhead et al. 2016, Chen et al. 2019b) in R v4.3 (https://www.r-project.org/). A relatively longer vector length indicates greater C limitation, and a vector angle <45° or >45° indicates the relative degrees of N or P limitation, respectively (Chen et al. 2019b).

2.5. Statistical analyses
ANOVA and Duncan's test were used to determine the significant differences among land-use types in the SPSS 18.0 (SPSS Inc., USA). The ηp² (partial eta-squared) statistic reported by two-way ANOVA was used to determine the relative importance of land use type and soil depth or interaction (Ding et al. 2019). Redundancy analysis (RDA) with forward selection was used to quantify the effect size of the key factors that shaped soil microbial element limitations in the Vegan package in R (Ding et al. 2020a), variables that had a variance inflation factor ≥ 4 were excluded to avoiding collinearity.

3. Results and discussions
Effect of land-use types on soil microbial element limitations. In general, C is the major limiting element for soil microbes in the subtropics of Southwestern China (Chen et al. 2019b). In our study, C limitation in the microbial communities was the lowest in SL (Figure 1a). The reason is that the vegetation from SL contains more annual herbs than GL and FL, which are more easily decomposed by microorganisms because annual herbaceous plants have higher carbohydrate and cellulose and lower lignin contents, and C resources from herbaceous plants are thus more easily exploited by microbes (Cui et al. 2019c). Two-way ANOVA showed land use type and vegetation type but not soil depth and their interaction had significantly impact on both C and NP limitations (Table 1), indicating land use type and vegetation type were the major forces driving C and NP limitations. N limitation
occurred in CL and FL, NP limitation occurred in SL and GL (Figure 1b), suggesting that microbial N limitation is more evident relative to microbial P limitation in the studied soils (Chen et al. 2019b). In addition, the N limitation in CL and FL is more serious than that in SL and GL (Figure 1b). Therefore, the conversion from natural vegetation to artificial vegetation would change microbial element limitations from C, N & P limitation to C & N limitation, and exacerbate N limitation.

![Figure 1](image1.png)

**Figure 1.** Soil microbial element limitations differ among land-use types. Note: CL—crop land; FL—afforested land; SL—shrub land; GL—grassland. Different lower-case letters in different columns indicate significant differences at P<0.05.

Environmental controls on soil microbial element limitations. The potential mechanisms control over the microbial C and NP limitations were grouped into four pathways that may operate simultaneously:

(a) Climate factors i.e., mean annual precipitation and mean annual temperature were found to collectively explained larger variation in microbial element limitations than plant or soil did across a precipitation gradient, thus the microbial C and NP limitations on regional scales were mainly controlled by climate factors (Cui et al. 2019b).

(b) Vegetation strongly compete with soil microbes for soil nutrients (Cui et al. 2019a, Ding et al. 2020a). Nutrient uptake by vegetation can reduce soil nutrients availability and hinder nutrient acquisition of soil microbes, thereby inducing soil microbial element limitation (Cui et al. 2019a).
(c) Soil directly supply for microbes with energy and nutrients (Cui et al. 2019b). Soil nutrient stoichiometry reflects nutrient balance (Ding et al. 2020a). An unbalance supply of soil nutrient would induce microbial element limitation. Therefore, soil elemental stoichiometry regulates the limitation of soil microbial elements (Cui et al. 2019a, Ding et al. 2020a).

(d) Previous studies found soil nutrient and physical property drove microbial element limitations (Cui et al. 2019a). Via excluding the insignificant factors, RDA in this study revealed that STN and pH significantly explained 41.543% of the variation in soil C limitation, STP and STN significantly explained 45.117% of the variation in soil NP limitations, STN was identified as the main factors affecting soil C limitation, STP was identified as the main factors affecting soil NP limitations (Table 2), supporting previous findings.

Table 1. Interactions and significance effect of Land use type and Soil depth on C limitation and NP limitation. The numbers outside the brackets were partial eta squared ($\eta^2$) which were used to determine the relative importance of each factor or interaction (a higher $\eta^2$ represents a greater importance), and the numbers inside the brackets were p value.

| Factors                      | C limitation | NP limitation |
|------------------------------|--------------|---------------|
| Model                        | 0.994(0.000) | 0.997(0.000)  |
| Soil depth                   | 0.221(0.050) | 0.132(0.183)  |
| Land use type                | 0.735(0.000) | 0.572(0.000)  |
| Soil depth * Land use type   | 0.310(0.143) | 0.086(0.886)  |
| Model                        | 0.984(0.000) | 0.997(0.000)  |
| Soil depth                   | 0.098(0.212) | 0.108(0.181)  |
| Vegetation type              | 0.346(0.000) | 0.476(0.000)  |
| Soil depth * Vegetation type | 0.087(0.256) | 0.025(0.687)  |

Table 2. Individual contributions of each significant factors to the percentage of variation (%) explained in soil microbial element limitations. The number outside each bracket is the percentage of variation in soil microbial element limitations. The number inside each bracket is the p value. The unexplained percentage is the variation not attributed to significant factors.

| Factors                      | VarC limitation | VarNP limitation |
|------------------------------|-----------------|------------------|
| STP                          | -               | 28.334(0.001)    |
| STN                          | 30.468(0.001)   | 16.783(0.003)    |
| pH                           | 11.075(0.019)   | -                |
| Unexplained                  | 58.457          | 54.883           |

4. Conclusions
Soil total N was the major factor affecting soil microbial C limitation.
Soil total P was the major factor affecting soil microbial NP limitations.

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