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Original article

Community size, activity and C:N stoichiometry of soil microorganisms following reforestation in a Karst region

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ABSTRACT

Reforestation has been widely adopted to restore soil fertility and ecosystem service in the rocky-desertified Karst region of southwestern China. As a key indicator of ecosystem restoration, soil microbial characteristics however remained poorly understood following reforestation on the degraded Karst soils. This study employed site comparisons along a chronosequence of *Toona sinensis* reforestation in soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), basal respiration (BR), microbial metabolic quotient (qCO_2), microbial quotient (MBC:TOC), and microbial C:N stoichiometry. Results showed that MBC, MBN, BR as well as soil organic C and N contents, moisture and porosity increased with the reforestation age. The 0-yr control of bare soil showed the highest microbial metabolic quotient (qCO_2, 2.48 mg g^{-1} h^{-1}) and lowest microbial quotient (MBC:TOC, 2.29%), reflecting the disturbed and stressed habitats. After 12-yr reforestation, qCO_2 decreased to 1.49 mg g^{-1} h^{-1} and MBC:TOC increased to 4.70%, due to the disturbance cessation and the weakened stress. The consistent increases in microbial C:N ratio and soil C:N ratio under the reforestation indicated microbial adaptive response to substrate resource stoichiometry. This study suggested that these microbial indices independently or in combination can be used as the early indicators of the restoration of Karst ecosystems.

1. Introduction

The Karst terrains formed from carbonate minerals account for about 15% of the world's land area [1]. The unique Karst ecosystem is very fragile and sensitive, with a low environmental capacity [2]. The soil is thin, coarse, highly erosive and degenerative [3]. The Karst soil covering approximately 0.55 million km^2 in southwest China, has been subjected to intensive anthropogenic disturbances (e.g., cultivation, deforestation, grazing and burning) [4,5]. These disturbances rapidly expanded after 1970s because of the pressure from the increasing population and land overuse [6,7], and therefore accelerate the Karst ecosystem degradation especially of rocky-desertification [8]. The degradation adversely affects soil fertility and thereby results in the genesis of abandoned bare land. Such degraded land needs proper ecological restoration through which soil can be ameliorated to support biological productivity [9]. Reforestation, as one method by which degraded sites can be restored back to maintain soil fertility [9], has been widely adopted in the Karst regions since 1990s. Previous studies indicate that soil physicochemical properties have been improved after the reforestation. Soil porosity, moisture, organic carbon (C) and nutrient contents significantly increased following the reforestation [2,10,11]. Higher values of soil C to nitrogen (N) ratio (15.2–19.5) were observed under various reforestations than under the control soil (10.1) [10]. However, soil microbial characteristics were relatively poorly known.

Soil microorganisms play an important role in regulating soil organic matter decomposition and nutrient cycling, consequently affecting ecosystem service functioning [12]. With high turnover rate, soil microorganisms generally are sensitive to environment

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changes and anthropogenic activities [13,14]. Soil microbial biomass and activity (e.g. basal respiration) have been extensively used as indicators for soil fertility and environment quality [15,16]. Soil microbial metabolic quotient and microbial quotient have been found to reflect stress effects, and therefore have been extensively used as eco-physiological indicators [16–18]. They also link microbial community structure and diversity [17,18]. The stoichiometry of soil microbial biomass is a field of growing interest because important ecosystem fluxes of C, N and P, such as mineralization or immobilization, are controlled by soil microorganisms [19,20]. Well constrained C:N:P ratios have been found for whole soil microbial communities. A mean global microbial biomass C:N:P ratio of 60:7:1 was reported in various soils [21], similar to the Redfield ratio described for planktonic biomass [22]. Yet, these concepts have been contradicted by studies showing stoichiometric variability in the soil microbial biomass (between 11:1:1 and 93:10:1) [23]. The stoichiometry ratio can be modified when a given nutrient becomes limiting [24]. Therefore, soil microbial stoichiometry can adaptively vary with resource stoichiometry. Information on these microbial properties is required for a better understanding of the phytoremediation mechanisms and the interactions between soil and plant community, and for the appropriate management and conservation of the Karst eco-environment.

The objective of this study was to examine how the selected soil microbial indices respond to the reforestation on the rocky-desertified Karst soil. We predicted that: i) soil microbial biomass and activity increase in response to the plantation forestry due to increased substrate resources (soil organic C and nutrient contents), and/or improved environmental conditions (soil moisture, porosity); ii) microbial quotient increases and metabolic quotient decreases after the plantation because of the weakened rocky-desertification stress and/or possibly increased fungi:bacteria ratio, and community diversity; iii) The stoichiometry for microbial components is plastic, and microbial biomass C:N ratio increase with increasing resource C:N ratio under the plantation.

2. Materials and methods

2.1. Study site and experimental design

This study was conducted at Huanjiang county (107°54′ E, 24°49′ N), Guangxi province in China, with typical Karst ecosystems. This region belongs to a subtropical monsoon climate with mean annual precipitation of 1389 mm and mean annual temperature of 19.9 °C. The calcareous soil developed from a dolostone base [25]. The soil degradation is serious due to the intensive anthropogenic disturbance especially of cultivation and therefore results in a large area of abandoned bare land in this region. Since 1990s, large scales of *Toona sinensis* forestation have been adopted to restore the degraded ecosystem. Four *T. sinensis* forestations (2-, 4-, 8-, and 12-year) were selected as the experimental sites while a nearby degraded bare land as the 0-year control site. The *T. sinensis* was planted at a 7 m × 5 m density. The tree diameter and height averaged 2 cm and 2.52 m, 5 cm and 2.54 m, 11 cm and 2.59 m, 16 cm and 2.64 m for the 2-, 4-, 8-, and 12-year forestations, respectively. The five sites were selected to show very similar altitude, topography, vegetation background and disturbance experience (over 40 years of cultivation) before forestation and the same soil type. Each site has a size of over 10,000 m^2, and six plots (20 m × 20 m) as replications were selected within each site for sampling.

2.2. Sampling and analysis

Ten soil cores (2.5 cm diameter) were collected at 0–10 cm depth and mixed to form one composite sample, after excluding litter and humus layer in each plot in June 2012. Porosity was determined using a core method, based on undisturbed soil [26]. Subsamples, after oven-dried at 60 °C and passed through 0.15 mm sieve, were used to measure soil total C (TC) and total N (TN) contents (using an element Analyzer (Elementar, Germany)), and total organic carbon (TOC) content (using the $K_2Cr_2O_7$ oxidation method [26]). Total inorganic C (TIC) content was difference between TC and TOC. The TOC to TN ratio (TOC:TN) was calculated. Moisture was measured by an oven-dry method, and expressed on mass basis of dry soil. The air-dried and 2 mm sieved subsamples were used to determine soil pH with a pH meter. Moist field subsamples, after passed through a 2 mm sieve, were used for analysis of moisture, microbial biomass C (MBC), and microbial biomass N (MBN) and basal respiration (BR). The MBC and MBN were measured by fumigation-extraction method [27]. The C and N contents, extracted with 0.5 M K_2SO_4 from the chloroform-fumigated and unfumigated moist samples, were estimated by an automated TOC-V-TN Aanalyzer (Analytik, Jena, Multi N/C 3000), using a conversion factor of 0.45 for MBC and 0.54 for MBN [27,28]. The percentage of TOC present as MBC was calculated as Microbial quotient (MBC:TOC). The percentage of TN present as MBN (MBN:TN) was also calculated. The BR was measured by alkali absorption of CO_2 evolved at 25 °C in dark for 14 days followed by titrating the residual OH^- with a standardized acid [29]. Microbial metabolic quotient (qCO_2) was calculated from the results of BR and MBC as follows: (CO_2–C evolved in 14 days kg^{-1} soil)/(microbial biomass C kg^{-1} soil)/(14 days × 24 h) × 1000, then qCO_2 was expressed as mg CO_2–C g^{-1} MBC h^{-1} [30]. All microbial indices were expressed on mass basis of oven-dried soil.

2.3. Statistical analysis

Responses of the measured soil properties to reforestation age, and correlations of MBC vs MBN, MBC:MBN vs TOC:TN, and MBC:MBN vs qCO_2, were evaluated by linear regression using SPSS 13.0 software package. The differences in all variables among the reforestation ages were assessed by one-way variance analysis (ANOVA) with a LSD test. To separate and evaluate effects of soil substrate resource (organic C and nutrients) and soil environmental conditions (moisture and porosity) on the selected soil microbial characteristics, canonical correspondence analysis (CCA)-based variation partitioning analysis (VPA) was performed by R language 2.13.1.

3. Results and discussion

3.1. Soil physicochemical properties

Soil porosity, moisture, TOC and TN contents all showed a significant linear increase with increasing the age of *T. sinensis* forestation, with mean annual increments of 1.09%, 0.61%, 0.49 g kg^{-1} and 0.05 g kg^{-1} respectively (Fig. 1a, b, d, f). The TOC:TN ratio (9.7–11.4) also tended to increase with the forestation age (Fig. 1g). Soil TC content (54.9–59.5 g kg^{-1}), TIC content (41.6–44.6 g kg^{-1}) and pH (6.72–6.91) showed no significant changes following the forestation (Fig. 1c, e, h).

The high TIC content and pH value showed the calcareous soil condition of the Karst region. Our results indicated that the *T. sinensis* forestation improved soil physiochemical fertilities. This is consistent with previous studies [2,10,11]. The *T. sinensis* forestation might increase soil aggregation via increasing binding agents of TOC, roots, microorganisms [10], and thus increased soil porosity. The improved pore status might enhance water holding capacity and infiltration [31] and therefore resulted in the increase of moisture. The increased moisture might also relate to the increased