Supplementary Materials

Response of Soil Microbial Community to C:N:P Stoichiometry along a *Caragana korshinskii* Restoration Gradient on the Loess Plateau, China

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Appendix 1-Method for the determination of soil microbial community properties

1.1. Soil DNA extraction, PCR amplification, and Illumina sequencing

Soil DNA was extracted in triplicate from 0.5 g of fresh sample using the FastDNA spin kit for soil (MP Biomedicals, Cleveland, USA), following the manufacturer's instructions. The quality and integrity of the DNA extracts were checked by 1.0 % agarose gel electrophoresis [1]. The V4 region of bacterial 16S rRNA genes were amplified using the primer combination of 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTC AATTCMTTTRAGTTT-3′) [2]. The fungal ITS-1 region was amplified by using fungi-specific primers ITS1F (5′-ACTTGGTCATTTAGAG-GAAGTAA-3′) and ITS2 (5′-BGCTGCGTTCTTCATCGATGC-3′) [3]. The primers were tagged with unique barcodes for each sample. The reaction system of bacterial PCR amplification contained 0.4 μl of each primer, 1.25 μl of template DNA (10 ng), 0.4 μl of FastPfu polymerase (Beijing TransGen Biotech Co., Ltd, China), 2.5 μl of 10 × PCR buffer, 2.0 μl of dNTPs (2.5 mM), 5.0 μl of 5 × high enhancer, and 13.45 μl of sterile ultrapure water, in a total reaction volume of 25 μl. The PCR amplification of 16S rRNA was performed at an initial denaturation temperature of 95 °C for 3 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s. A final elongation step at 72 °C was run for 10 min. The PCR reaction system of ITS gene was also performed in a 25 μl mixture, which contained 0.5 μl of the two primers (30 μmol μl^{-1}), 1.5 μl of template DNA (10 ng), and 22.5 μl of Platinum PCR SuperMix (Invitrogen, Shanghai, China). The thermal cycling program consisted of an initial annealing temperature at 95 °C for 2 min, followed by 30 cycles with denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s and final extension at 72 °C for 5 min. The three replicated amplification products of bacteria and fungi in each soil sample were mixed to provide one final PCR product. Each mixed PCR product was purified using the Qiagen™ PCR purification kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol, and then were eluted in sterile water. Subsequently, 2.0 % agarose gel electrophoresis were used to quantified the concentration of each mixed gene (16S rRNA gene and ITS rRNA gene). Finally, the purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform (Personal, Shanghai, China) according to the standard protocols.

1.2. Sequence processing

Raw FASTQ files of sequencing were de-multiplexed and quality-filtered using Quantitative Insights Into Microbial Ecology (QIIME, Version 1.9.0) workflow [1]. Briefly, tags sequence, primer sequence, sequences <200 bp, an average quality score of <25 and reads containing ambiguous bases or any unresolved nucleotides were removed to obtain the high-quality sequence for subsequent analysis (Caporaso et al., 2010). Forward and reverse reads were incorporated into full-length sequences with FLASH (Fast Length Adjustment of Short reads). The above processed sequences

were clustered into operational taxonomic units (OTUs) defined by 97 % similarity using UPARSE (version 7.1) program [4], and chimeric sequences were identified and removed using UCHIME [5]. Subsequently, the largest number of sequences in each OTU were selected as the representative sequence to prepare OTUs table [6]. For bacteria, the OTU representative sequences were assigned using the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/index.jsp) classifier to identify bacterial taxonomies with a confidence threshold of 0.8 [7], whereas the representative sequences of fungi were identified using the UNITE database (https://unite.ut.ee/repository.php) with the lowest similarity of 0.8 [9]. Python Nearest Alignment Space Termination (PyNAST) workflow was applied to align the representative sequence to the reference sequence [8] and phylogenetic analysis of 16S and ITS sequences were conducted by Fast tree software [9]. The complete dataset was sent to the Sequence Read Archive (SRA) database of the NCBI under the accession numbers of SRP223565 for bacteria and SRP223564 for fungi.

1.3. Data processing

After quality sequencing, both bacterial diversity (544,702 high-quality sequences) and fungal diversity (919,779 high-quality sequences) were obtained with the 515F/907R (bacterial 16S rRNA) and ITS1F/ITS2 (fungal ITS) primer sets across all soil samples, respectively. The number of bacterial sequences varied from 32,064 to 56,602 per sample (mean=45,391), whereas the number of fungal sequences varied from 55,815 to 133,136 per sample (mean=76,648). To minimize any bias in the distribution of taxa, bacterial and fungal diversities of each treatment were calculated based on randomly selected sequences until the count reached saturation in the rarefaction curves. For the downstream analysis of bacteria, datasets were rarefied to 32,000 sequences, whereas for the downstream analysis of fungi, datasets were rarefied to 55,000 sequences. The OTUs were clustered at a similarity above 97 % to calculate the diversity indices.

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Table S1. Relative abundance of the dominant groups of soil microbial community at the phylum level following afforestation.

Restoration stages	*Proteobacteria*	*Actinobacteria*	*Acidobacteria*	*Chloroflexi*	*Gemmatimonadetes*	*Nitrospirae*	*Bacteroidetes*	*Verrucomicrobia*	*Planctomycetes*	*Others*
FL	25.16(2.51) ^{b}	41.19(2.77) ^{a}	13.76(0.87) ^{c}	6.31(0.39) ^{NS}	7.77(0.17) ^{NS}	1.24(0.06) ^{b}	1.34(0.14) ^{b}	0.77(0.03) ^{NS}	0.35(0.05) ^{b}	2.11(0.22) ^{ab}
CK17	25.54(0.69) ^{b}	33.76(1.50) ^{b}	17.06(0.57) ^{ab}	6.96(0.70)	9.13(0.71)	1.91(0.11) ^{a}	1.65(0.03) ^{b}	1.01(0.58)	0.80(0.31) ^{ab}	2.18(0.36) ^{ab}
CK32	31.53(0.57) ^{a}	26.07(1.40) ^{c}	17.88(0.32) ^{a}	6.36(0.37)	7.98(0.43)	2.09(0.29) ^{a}	3.03(0.22) ^{a}	1.36(0.36)	1.10(0.17) ^{a}	2.60(0.20) ^{a}
CK42	34.78(1.27) ^{a}	26.66(0.60) ^{c}	15.42(0.49) ^{bc}	6.17(0.62)	7.95(0.36)	2.45(0.13) ^{a}	3.46(0.22) ^{a}	0.92(0.19)	1.05(0.09) ^{a}	1.48(0.16) ^{b}

	Ascomycota	*Basidiomycota*	*Zygomycota*	*Others*
FL	75.58(2.50) ^{NS}	7.02(0.76) ^{c}	10.75(1.85) ^{a}	6.64(1.12) ^{NS}
CK17	71.96(1.16)	4.12(0.68) ^{c}	13.42(1.76) ^{a}	10.49(0.24)
CK32	71.39(3.15)	13.93(0.65) ^{a}	4.06(0.39) ^{b}	10.61(2.45)
CK42	68.37(2.46)	10.84(1.36) ^{b}	10.74(2.25) ^{a}	10.05(2.38)

The data for the average relative abundances from three replicates were calculated as the ratio between the abundance of the sequence type and the total number of sequences. All calculations used normalized data. Means with different lowercase letters indicate significant differences among different stages of vegetation restoration based on one-way ANOVA followed by an LSD test ($p < 0.05$).

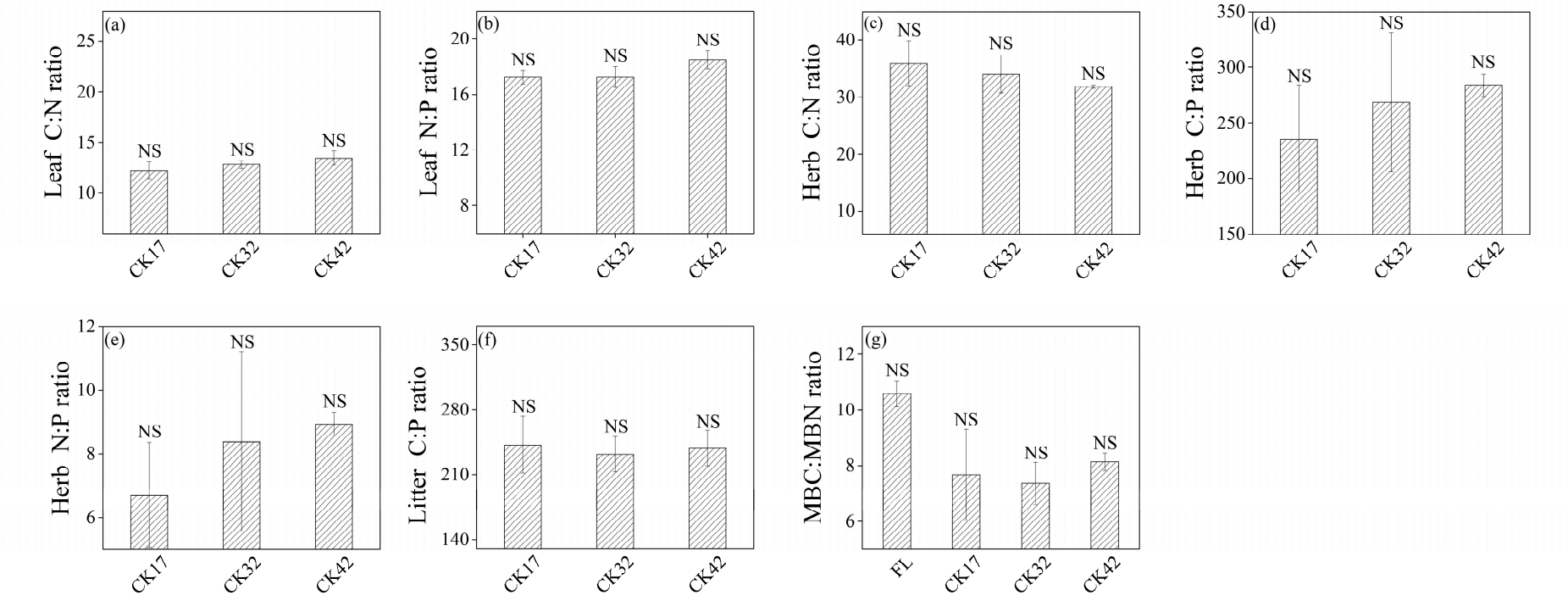

Figure S1. Changes of C, N, P stoichiometry in leaf (a,b), herb (c,d,e), litter (f), and microbial biomass (g) among different stages of vegetation restoration. Error bars indicate the standard errors of the means. Means with different lowercase letters indicate significant differences among different stages of vegetation restoration based on one-way ANOVA followed by an LSD test ($P < 0.05$).