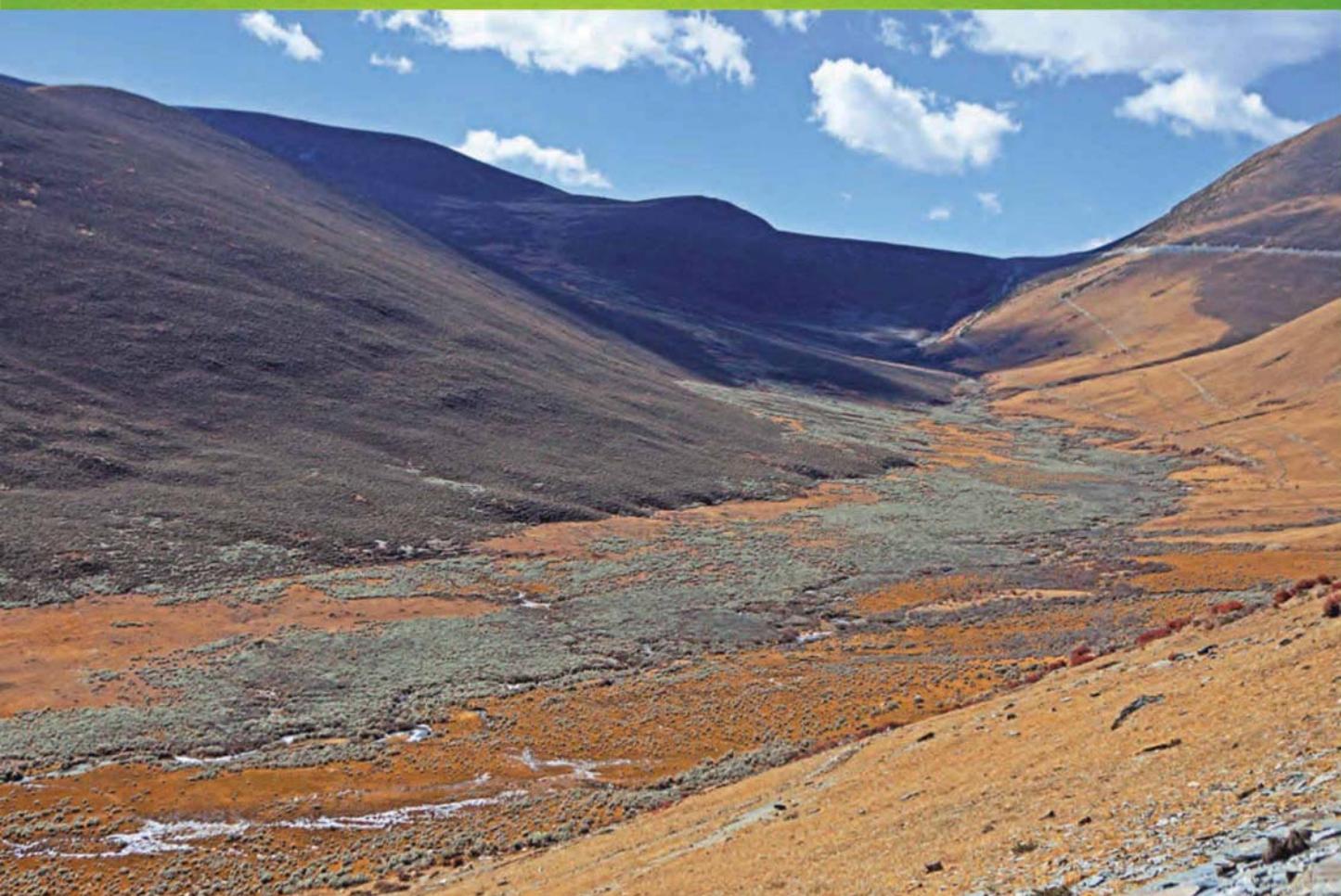


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封面图说: 川西高山地带土壤及植被——青藏高原东缘川西的高山地带坡面上为草地, 沟谷地带由于低平且水分较充足, 生长有很多灌丛。川西地区大约在海拔 4000m 左右为林线, 以下则分布有亚高山森林。亚高山森林是以冷、云杉属为建群种或优势种的暗针叶林为主体的森林植被。作为高海拔低温生态系统, 高山-亚高山地带土壤碳被认为是我国重要的土壤碳库。有研究表明, 易氧化有机碳含量与海拔高度呈显著正相关, 显示高海拔有利于土壤碳的固存。因而, 这里的表层土壤总有机碳含量随着海拔的升高而增加。

彩图及图说提供: 陈建伟教授 北京林业大学 E-mail: cites.chenjw@163.com

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邢肖毅, 黄懿梅, 安韶山, 闫浩. 黄土丘陵区不同植被土壤氮素转化微生物生理群特征及差异. 生态学报, 2013, 33(18): 5608-5614

Xing X Y, Huang Y M, An S S, Yan H. Characteristics of physiological groups of soil nitrogen-transforming microbes in different vegetation types in the Loess Gully region, China. Acta Ecologica Sinica, 2013, 33(18): 5608-5614.

黄土丘陵区不同植被土壤氮素转化 微生物生理群特征及差异

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摘要:采用最大或然计数法(most probable number, MPN)对黄土高原洞子沟流域不同植被恢复阶段土壤氮素微生物生理群(氨化细菌、亚硝化细菌、反硝化细菌)数量分布特征进行了测定,结果表明:1)土壤氨化细菌、亚硝化细菌和反硝化细菌数量随植被恢复而增加,三者最大值分别为最小值的74.4和31倍,其中氨化细菌和反硝化细菌的数量在铁杆蒿群落最低,辽东栎群落最高,亚硝化细菌数量在丁香群落最低,辽东栎群落最高;2)植被恢复对各氮素生理群影响不同,对氨化细菌影响最大,其次分别为反硝化细菌和亚硝化细菌;3)各氮素生理群数量差异较大,氨化细菌>反硝化细菌>亚硝化细菌。研究区氨化细菌占总数的75%—80%,反硝化细菌占20%—25%时,生态系统最为稳定;4)土壤理化性质与各功能菌关系紧密,其中,土壤容重和硝态氮含量与微生物数量相关性最大,全钾、矿化氮和微生物量氮也表现出很大的相关性。

关键词:黄土沟壑区;植物群落;氨化细菌;亚硝化细菌;反硝化细菌

Characteristics of physiological groups of soil nitrogen-transforming microbes in different vegetation types in the Loess Gully region, China

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Abstract: The “Grain for Green” project is one of the important measures used to improve the environment of China’s Loess Plateau. In recent years, soil physicochemical properties in the region have been improved, which in turn promoted ecological succession. Different vegetation types change the soil environment to different extents, influencing the biogeochemical cycling of materials such as nitrogen, an essential nutrient for plants. Recently, a growing amount of attention has been paid to the relationship between vegetation and nitrogen cycling in natural ecological systems. Meanwhile, several physiological groups of nitrogen-transforming microbes in soil are closely associated with soil nitrogen cycling. To determine the effect of vegetation restoration (grass stage, shrub-grass stage and tree-shrub-grass stage) on the populations and distribution of physiological groups of nitrogen-transforming microbes (ammonifying bacteria, nitrifying bacteria and denitrobacteria), soil samples were collected from depths of 0—10 cm under seven vegetation types from different stages of restoration in the Dongzigou Basin, Ansai, Shaanxi Province and bacteria populations were measured through the most-probable-number (MPN) method. The results show the populations of ammonifying bacteria, nitrifying bacteria, and denitrobacteria increased as vegetative succession proceeded from grass communities to more complex tree-shrub-grass

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communities, and those populations ranged from 2.49 to 185.79×10^6 cfu/gdm, 19.43 to 77.21×10^2 cfu·g⁻¹·dm⁻¹ and 1.23 to 38.24×10^6 cfu·g⁻¹·dm⁻¹, respectively. For ammonifying bacteria and denitrobacteria, the highest N levels were obtained in the *Quercus liaotungensis* Koidz. community and the lowest in the *Artemisia sacrorum* Ledeb. community. The maximum population levels of denitrobacteria and ammonifying bacteria were obtained in the *Q. liaotungensis* Koidz. community and these ranged between 31 to 74 times higher than the lowest levels which were seen in the *Artemisia sacrorum* Ledeb. community, respectively. The highest levels of nitrifying bacteria were obtained in the *Q. liaotungensis* community and these were 4-fold higher than the lowest one in the *Syzygium aromaticum* (L.) Merr. and Perry community. The effect of vegetation restoration on the population of nitrobacteria was the strongest on ammonifying bacteria, followed by denitrobacteria and nitrifying bacteria. Soil nitrogen bacteria groups varied sharply from group to group, forming a decreasing order of ammonifying bacteria > denitrobacteria > nitrify bacteria. In study area, ammonifying bacteria accounted for about 75%—80% of the sum of ammonifying bacteria, nitrifying bacteria and denitrobacteria, while when denitrobacteria accounted for 20%—25% of that sum; this indicates that the ecosystem was relatively stable. Soil physicochemical properties were closely related to soil nitrogen content and the abundance of nitrogen bacteria groups. Soil bulk density had a larger impact on soil nitrogen bacteria groups than other soil physicochemical properties, similar to the relationship of nitrate nitrogen in soil and various forms of nitrogen in soil. Total potassium, mineralizable nitrogen and microbial biomass nitrogen content also had a major impact on soil nitrogen bacteria groups. In general, microbes are more responsive to changes in plant communities than are soil physical and chemical properties. Studying the characteristics of microbial communities, especially functional microbes that modify the availability of key soil nutrients, e.g. nitrogen, provides significant data which are useful in estimating the ecological benefits of restoring vegetative communities. Therefore, more detailed research studies in this field are needed in the future.

Key Words: Loess Gully; vegetation types; ammonifying bacteria; nitrify bacteria; denitrobacteria

黄土高原是我国水土流失最严重地区的典型代表,退耕还林还草是解决该区水土流失问题的必然选择。随着退耕还林还草工作的开展,土壤质量逐渐改善^[1],退耕区及周边的整个生态环境质量得以改善^[2]。目前,很多学者对植被恢复效果进行了评价,其研究主要集中于土壤理化性质及土壤养分方面^[1,3],而从土壤微生物群落角度探讨的研究较少^[4-6],氮素微生物生理群则更为鲜见。土壤微生物是土壤中有生命的成分,对土壤各种变化极为敏感,能充分反映土地利用方式和生态功能的变化^[7],对维持生态系统氮循环极其重要^[8]。土壤氮素转化生理群包括氨化细菌、硝化细菌、自生固氮菌和反硝化细菌,其数量和活性可以反映所处生境氮素物质的水平及转化规律,关系到土壤生态系统的维持和稳定^[9]。目前,对氮素生理群微生物的研究多见于农地和草地,且重在探讨人为干扰^[10-13]对其产生的影响,对自然恢复条件下土壤的研究很少。探究不同植物群落下土壤氮素微生物生理群数量的变化规律,可以揭示黄土高原地区地下生理生态格局,对探明土壤氮素转化的微生物机制及植被恢复对土壤生态环境的改善效果具有重要意义。

1 材料与方法

1.1 野外土壤样品采集

1.1.1 研究区域概况

洞子沟地处安塞县南部楼坪乡,109°7'34"—109°10'34"E,36°31'13"—36°35'26"N,海拔1166—1490 m,流域总面积20.61 km²。该区属于中温带大陆性半干旱季风气候,四季长短不等,干湿分明。年平均气温8.8 ℃,年平均降水量505.3 mm,年日照时数为2395.6 h,日照百分率达54%,总年无霜期157 d。土壤类型以黄绵土为主,土地利用以林地为主。

1.1.2 供试土壤样地植被情况

供试土壤取自洞子沟流域不同恢复阶段(草本、灌草、乔灌草)的7种典型植被群落。根据各个样地主要建群种名称,样点依次记录为辽东栎(*Quercus liaotungensis*),三角槭(*Acer buergerianum* Miq.),侧柏(*Platycladus orientalis*),丁香(*Syzygium aromaticum*),狼牙刺(*Sophora davidii*),茭蒿(*Artemisia giraldii* Pamp.),铁杆蒿(*Artemisia sacrorum* Ledeb.)。采样点地理信息和植被情况如表1所示。

1.1.3 样品采集方法

供试土壤采集于2011年7月,在选定区域内根据植被类型确定样方大小(乔木10 m×10 m,灌木5 m×5 m,草地2 m×2 m),每个样地在野外设置3个重复,即设置3个样方,分别相隔约100 m²。每个样方内以S形选取5个点,去除土壤上层枯落物,用

土钻采集0—10 cm土壤样品,将采集的土样混合,密封后带回实验室内,仔细除去其中可见植物残体及土壤动物,过2 mm筛,测定氮素微生物生理群数量。

表1 采样点地理信息和植被情况

Table 1 Geographic information and vegetation summarize of the sampling sites

植被类型 Vegetation types	样地名称 Sample name	经度/(°) Longitude	纬度/(°) Latitude	海拔/m Altitude	坡向 Slope aspect	坡度/(°) Slope gradient	坡位 Slope position	群落名称 Vegetation community
乔灌草 Arbor-brush-grass	辽东栎	109.174E	36.555N	1259	阴坡	28	下	辽东栎+土庄绣线菊、丁香、多花胡枝子+披针苔草
	三角槭	109.153E	36.575N	1334	阴坡	21	中	三角槭+黄刺玫、黑榆子+披针苔草
	侧柏	109.163E	36.556N	1301	阳坡	30	下	侧柏+六道木、丁香+披针苔草
灌草 Brush-grass	丁香	109.162E	36.570N	1284	阳坡	35	中	丁香、虎榛子、黄刺玫+茭蒿、铁杆蒿
	狼牙刺	109.166E	36.574N	1346	阳坡	27	下	狼牙刺+白羊草、达乌里胡枝子
草本 Grass	茭蒿	109.158E	36.582N	1348	阴坡	25	中	茭蒿、铁杆蒿
	铁杆蒿	109.158E	36.582N	1351	阴坡	30	上	铁杆蒿

辽东栎 *Quercus liaotungensis*; 三角槭 *Acer buergerianum* Miq; 侧柏 *Platycladus orientalis*; 丁香 *Syzygium aromaticum*; 狼牙刺 *Sophora davidii*; 茭蒿 *Artemisia giraldii* Pamp; 铁杆蒿 *Artemisia sacrorum* Ledeb

1.2 测定指标及其方法

1.2.1 氮素微生物生理群数量的测定

土壤氨化细菌、亚硝化细菌、反硝化细菌数量采用最大或然计数法(MPN)进行测定。氨化细菌采用蛋白胨琼脂培养基,亚硝化细菌采用改良的斯蒂芬逊培养基,反硝化细菌采用组合培养基,以稀释法测定其数量^[14]。MPN方法在测定细菌数量时虽不够精确^[15],但所有样品同一步骤测定,结果仍具有一定的可比性。

1.2.2 土壤基本理化性质及各形态氮素含量的测定

土壤理化性质及各形态氮素含量均采用常规方法,见《土壤农化分析》^[16]。

1.3 数据处理方法

试验数据处理采用Microsoft Excel 2003和SPSS18.0软件。多重比较采用LSD方法,相关分析采用Pearson相关分析法。

2 结果与分析

2.1 土壤基本理化性质及氮素含量

研究区土壤呈弱碱性,从草本群落到乔灌草群落,土壤pH值和容重均逐渐降低,最大值分别比最小值高出了7.7%和42.3%。土壤有机质、全氮、全磷、硝态氮、铵态氮、可矿化氮(好气培养14d)、微生物量氮含量随着植被的恢复逐渐增加,最大值分别是最小值的11.1、9.20、1.31、27.9、2.35、8.70和6.46倍,硝态氮在含量不同植被群落下变异最大,即对植被变化最为敏感。土壤C/N介于8.56—15.9之间,变化呈现波动性。全钾含量较稳定,最大值和最小值相差仅2.64 g/kg。

表2 不同植被群落下土壤基本理化性质及氮素含量

Table 2 Soil physicochemical properties and nitrogen contents under different vegetation types

植被类型 Vegetation types	土壤pH Soil pH	容重 BD /(g/cm ³)	有机质 SOM /(g/kg)	全氮 Total N /(g/kg)	土壤C/N Soil C/N	全磷 Total P /(g/kg)	全钾 Total K /(g/kg)	硝态氮 Nitrate N /(mg/kg)	铵态氮 Ammonium N /(mg/kg)	可矿化氮 Available N /(mg/kg)	微生物量氮 MBN /(mg/kg)
辽东栎	8.18f	0.78b	71.5a	3.22a	12.9b	0.75a	21.1a	53.2a	2.89b	101a	129a
侧柏	8.34e	0.83b	59.5b	2.81b	12.3b	0.72a	19.5ab	49.3a	3.17a	70.1b	1046a
三角槭	8.48d	1.01a	54.5b	2.69b	11.8b	0.76a	19.8ab	21.0b	2.66b	65.9b	64.81b
丁香	8.64c	1.05a	31.19c	1.22c	14.8a	0.71a	18.6b	10.6c	2.42b	36.2c	42.2c
狼牙刺	8.81a	1.14a	11.1de	0.66d	9.72c	0.62b	18.9b	8.05d	2.56b	19.6e	41.8c
茭蒿	8.70b	1.07a	18.4d	0.85d	12.6b	0.61b	19.4ab	11.0c	1.93c	27.4d	37.4c
铁杆蒿	8.76b	1.11a	6.46e	0.35e	14.5a	0.58b	18.5b	1.91e	1.35d	11.6e	20.0d

BD: 容重 Bulk density; SOM: 有机质 Soil organic matter; MBN: 微生物生物量氮 Microbial biomass nitrogen; 不同字母表示同一土层总氮在不同植被群落下差异显著($P < 0.05$)

2.2 土壤氮素转化微生物生理群

从草本群落到乔灌草群落,3种氮素微生物生理群数量均增加。氨化细菌和反硝化细菌数量均表现为辽东栎群落最大,铁

杆蒿群落最小。氨化细菌最大值是最小值的 74.6 倍,反硝化细菌为 31.1 倍。亚硝化细菌最大值和最小值分别为辽东栎群落和丁香群落,相差 2.97 倍。可见,氨化细菌对植被变化最为敏感,反硝化细菌次之,亚硝化细菌最不敏感。3 种微生物数量差异很大,氨化细菌最多,平均占总数的 83%,其次是反硝化细菌,占 17%,亚硝化细菌最少。同一植被类型内各类微生物占总数的比例不同。两种草本群落相比,3 种微生物所占比例变化较大,以氨化细菌为例,茭蒿群落为 90.9%,而铁杆蒿群落仅为 66.8%,亚硝化细菌和反硝化细菌变化更大。灌草群落氨化细菌比例明显增加,反硝化细菌明显减小,两个样地间差别不大。乔灌草群落 3 个样地相比,各微生物占总数比例差异最小,其中,氨化细菌所占比例较灌草群落有所下降,而反硝化细菌有所增加。

表 3 不同植被类型下土壤微生物氮素生理群数量

Table 3 Soil nitrogen bacteria group communities under different vegetation types

植被类型 Vegetation types	氨化细菌($\times 10^6$) Ammonifying bacteria /(cfu·g ⁻¹ ·dm ⁻¹)	占总数百分比 Percentage of total/%	亚硝化细菌($\times 10^2$) Nitrify bacteria /(cfu·g ⁻¹ ·dm ⁻¹)	占总数百分比 Percentage of total/%	反硝化细菌($\times 10^6$) Denitrobacteria /(cfu·g ⁻¹ ·dm ⁻¹)	占总数百分比 Percentage of total/%
辽东栎	186a	82.9	77.2a	0.0034	38.2a	17.1
三角槭	137b	80.7	57.3b	0.0034	32.8a	19.3
侧柏	74.0c	73.8	50.2c	0.0050	26.3b	26.3
丁香	55.3bc	97.1	19.4d	0.0034	1.66d	2.92
狼牙刺	48.8bc	95.2	24.5d	0.0048	2.44c	4.76
茭蒿	27.0c	90.9	24.4d	0.0082	2.69c	9.08
铁杆蒿	2.49d	66.8	25.1d	0.0671	1.23d	33.0

2.3 氮素微生物生理群数量与理化因子及氮素含量的相关分析

土壤理化性质、土壤氮素及氮素微生物生理群之间密切相关。氨化细菌、亚硝化细菌和反硝化细菌与土壤 pH 值及土壤容重显著或极显著负相关,与土壤有机质、全氮、全磷、全钾、硝态氮、可矿化氮及微生物量氮含量显著或极显著正相关,与 C/N 不相关。对于铵态氮,仅氨化细菌与其显著正相关,亚硝化细菌和反硝化细菌与之不相关(表 4)。

表 4 氮素微生物数量与理化因子及氮素含量简单相关分析

Table 4 Correlation coefficients of nitrogen bacteria groups and physicochemical properties and N contents

相关系数 Correlation coefficients	氨化细菌 Ammonifying bacteria	亚硝化细菌 Nitrify bacteria	反硝化细菌 Denitrobacteria
土壤 pH Soil pH	-0.82 *	-0.92 **	-0.77 *
容重 BD	-0.90 **	-0.92 **	-0.83 *
有机质 SOM	0.77 *	0.88 **	0.76 *
全氮 Total N	0.80 *	0.90 **	0.81 *
土壤 C/NSoil C/N	-0.26	-0.09	-0.26
全磷 Total P	0.74 *	0.72 *	0.74 *
全钾 Total K	0.83 *	0.91 **	0.80 *
硝态氮 Nitrate N	0.89 **	0.92 **	0.82 *
铵态氮 Ammonium N	0.79 *	0.67	0.69
可矿化氮 Available N	0.84 **	0.93 **	0.80 *
微生物量氮 MBN (SMN)	0.87 **	0.92 **	0.77 *

* * $P<0.01$, * $P<0.05$; BD: 容重 Bulk density; SOM: 有机质 Soil organic matter; MBN: 微生物生物量氮 Microbial biomass nitrogen

3 讨论

3.1 植被恢复对土壤理化性质及氮素含量的影响

黄土丘陵区自 20 世纪 90 年代施行退耕还林还草以来,随着植被恢复的进行,植被覆盖度逐渐增加,土壤质量逐渐改善^[17],氮素含量增加^[18]。主要原因有以下几点:一是植物生长过程中通过凋落物和分泌物向土壤中返还大量无机营养元素^[1,3];二是植物通过凋落物覆盖和根系的生长减少土壤侵蚀和水分蒸发;三是植物根系的生长穿插改善了土壤的通气状况,四是植物残体腐解过程中产生的酸类物质促进土壤中难溶物质向有效态转化^[19]。研究者们普遍发现,不同形态氮素对植被恢复的敏感性不同,一般认为,速效氮较全氮敏感^[1,3]。采样季节研究区域降雨较多,虽氨化作用强烈^[19],但铵态氮一方面易于转化为硝态氮,另一方面,易于被微生物摄取^[20],最终导致硝态氮含量升高较多,而铵态氮升高较少。

3.2 植被恢复对土壤氮素转化微生物生理群数量的影响

如上所述,黄土丘陵区植被恢复促进了土壤质量的提高,一方面,植物返还的营养物质为微生物提供了能源物质^[1,3],另一方面,土壤质地和通气状况的改善为微生物创造了较为有利的生长繁殖环境^[21],因此,氨化细菌和硝化细菌数量增加^[22],而硝化作用为反硝化作用提供了反应物硝态氮,有利于反硝化作用的进行,反硝化细菌数量增加^[23]。因此,3种微生物数量随着植被恢复逐渐增加,与前人的研究相符^[24]。黄土高原森林区植被的演替序列为草本群落-灌草群落-乔灌草群落^[25],随着演替的进行,生态系统稳定性提高^[2]。草本阶段微生物种群易于变化。灌草阶段,由于土壤中有机物质增多,土壤理化性质改善,氨化细菌数量增加,反硝化细菌数量较少。顶级乔灌草群落,林内郁闭度较高,光照不充足,土壤含水量升高,氨化细菌比例降低,占总数的75%—80%,反硝化细菌比例增加,占20%—25%。此时,生态系统最为稳定。

植被恢复对不同微生物的影响不同,对氨化细菌的影响最大,其次是反硝化细菌,亚硝化细菌最小。姚拓等发现扰动对草地硝化细菌的影响最大,反硝化细菌和氨化细菌次之^[26]。这说明土壤的退化和恢复的微生物机制不同,可以推测,退化首先表现为硝化细菌的减少,而恢复则是首先是氨化细菌的增加。然而,由于土壤微生物各生理群的分布数量等受多种因素的影响,例如土壤环境、植被状况、气候特点等^[27-28],因此上述推测还需进一步论证。研究区域3种氮素微生物生理群的数量差异较大,表现为氨化细菌和反硝化细菌较多,而亚硝化细菌数量较少,与前人的研究结果一致^[10,12]。这是因为植物将大量有机物返还给土壤,为氨化作用提供了丰富的基质,促进了氨化细菌的生长繁殖,而土壤中大量的易溶性有机物(根系分泌物、凋落物)消耗了氧气^[29],同时研究区域土壤呈碱性,为反硝化细菌提供了良好的环境条件^[30]。亚硝化细菌含量不高,一方面可能是因为植物根系分泌物种的酚类物质和有机酸会抑制硝化作用^[31]。另一方面,研究区域土壤属弱碱性,此时,硝化反应的第一步,即氨转化为亚硝酸的速度很快,而亚硝酸转化为硝酸的速度很慢^[32]。

研究区域土壤中铵态氮的累积量并不多,硝态氮的累积量却较大。这是因为铵态氮既参与后期的硝化作用,又易于被微生物吸收利用^[20],而硝态氮含量高可能有以下几方面的原因,一是土壤中硝态氮并不都是通过铵态氮的进一步硝化而产生的,土壤中存在着将有机氮直接转化为亚硝态氮的异养型微生物^[33-34]。二是土壤中硝态氮的积累量并不一定与硝化细菌的活动强度相一致^[35],刁治民发现高寒草地硝酸盐在夏季会出现积累现象^[36]。

3.3 植被恢复过程中影响氮素转化微生物生理群的关键因素

氮素微生物生理群与土壤基本理化性质和土壤氮素的相关分析表明,除C/N和铵态氮外,其他理化因子和氮素均与3种氮素微生物显著或极显著相关。这是由于植被恢复改善了土壤理化性质,增加了氮素含量,刺激了微生物的生长,因此微生物数量与理化指标、氮素表现出了很好的相关性,理化性质中以容重,氮素中以硝态氮对微生物数量影响最大,全钾、矿化氮和微生物量氮也有较大影响。3种微生物数量和容重均极显著负相关。研究区域土壤C/N较低,土壤氮素供给相对充足^[37],同时,温度、水分、土壤本身差异不大,因此,氧气含量就成了影响微生物数量的关键因素。氨化细菌和硝化细菌只有在好氧条件下才能发挥作用^[38],因此容重越小,土壤通气性相对越好,微生物数量相应增加。反硝化作用是在嫌气条件下进行的微生物过程,O₂留存不利于其生长。而反硝化细菌数量同样与容重负相关,说明较高的硝酸盐和易溶性有机物(根系分泌物、凋落物)对反硝化细菌的有利作用^[23]大于O₂的不利影响。王小纯等发现土壤中硝态氮的增加可以显著提高氨化细菌的活性^[39]。齐文娟也发现氮素是微生物的重要影响因子,与微生物数量正相关^[40]。而硝态氮是土壤中对植被恢复最为敏感的氮素,矿化氮、微生物量氮均属活性较大的有机氮组分,因此其均与3种氮素微生物正相关。有研究认为,土壤钾含量与微生物数量正相关^[40],其影响机制有待于深入研究。

4 结论

从草本群落到辽东栎群落,土壤质量得以改善,氮素含量逐渐增加,氨化细菌、亚硝化细菌和反硝化细菌数量增加,表现为乔灌草群落>灌草群落>草本群落。植被恢复对3种微生物的影响程度各不相同,对氨化细菌影响最大,反硝化细菌次之,亚硝化细菌最小。3种氮素微生物生理群的数量差异较大,氨化细菌>反硝化细菌>亚硝化细菌。当植被恢复至乔灌草相结合的较为稳定的阶段时,氨化细菌占总数的75%—80%,反硝化细菌占20%—25%左右。

容重、硝态氮和氮素微生物生理群数量关系最为密切,土壤通气性是影响微生物生长的重要因素,氮素微生物生理群的生长首先促进了植物有效氮含量的升高。另外,全钾、pH值、总氮、矿化氮和微生物量氮同样与氮功能微生物关系紧密。

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