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有机肥和生物炭添加对亚热带人工草地土壤微生物碳、磷限制的缓解作用

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摘要: 为了探究施肥对亚热带人工草地土壤酶活性以及酶化学计量特征的影响, 通过施加有机肥和生物炭, 探究土壤理化指标的变化以及对土壤酶活性及酶化学计量指标的影响, 并通过土壤胞外酶活性计量特征来评估微生物养分限制。结果表明, 施加有机肥和生物炭显著增加亚热带人工草地土壤有机碳、全氮含量和pH ($P < 0.05$)。施加有机肥显著增加 0~10 cm、10~20 cm、20~30 cm 土层 β -葡萄糖苷酶活性 (βG) 36.18%、37.21%、59.30% ($P < 0.05$), 增加 0~10 cm、10~20 cm、20~30 cm 土层 β -1,4-N-乙酰葡萄糖苷酶活性 (NAG) 21.16%、17.25%、30.24% ($P < 0.05$), 生物炭显著增加各土层亮氨酸氨基肽酶 (LAP) 和 NAG 活性 ($P < 0.05$)。亚热带人工草地土壤 C:N:P 获取酶的平均比例为 1:1.31:1.72, 偏离了 1:1:1, 表明微生物受磷限制。施肥后矢量长度、矢量夹角降低, 说明施肥可以缓解碳、磷限制。研究结果为亚热带人工草地改良与管理提供理论依据, 适当施加有机肥或生物炭, 有助于亚热带人工草地的改善与恢复, 以缓解碳、磷限制。

关键词: 土壤酶活性; 土壤酶化学计量; 养分限制; 有机肥; 生物炭

Mitigation of soil microbial carbon and phosphorus limitations through organic fertilizer and biochar inputs in subtropical cultivated grassland

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Abstract: The aim of this research was to investigate the effects of fertilization on soil enzyme activities and soil enzyme stoichiometry in subtropical cultivated grassland. We conducted a field experiment in which organic fertilizer and biochar were applied to subtropical grassland. Then, changes in soil physicochemical properties were monitored and the impacts of these inputs on soil enzyme activities and enzyme stoichiometric indices were determined. Additionally, microbial nutrient limitations were assessed through determination of the stoichiometric characteristics of soil extracellular enzyme activities. The results show that the application of organic fertilizer and biochar to

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subtropical cultivated grassland significantly increased the soil organic carbon and total nitrogen contents, as well as soil pH ($P < 0.05$). The application of organic fertilizer significantly increased β -1,4-glucosidase activity in the 0–10 cm, 10–20 cm, and 20–30 cm soil layers by 36.18%, 37.21%, and 59.30%, respectively ($P < 0.05$). It also increased β -1,4-N-acetyl aminidase activity in the 0–10 cm, 10–20 cm, and 20–30 cm soil layers by 21.16%, 17.25%, and 30.24%, respectively ($P < 0.05$). Application of biochar significantly increased leucine aminopeptidase and β -1,4-N-acetyl aminidase activity in all three soil layers ($P < 0.05$). The average ratio of C:N:P for enzyme acquisition in subtropical cultivated grassland soil was 1:1.31:1.72, deviating from 1:1:1, indicating that microorganisms in this area are phosphorus-limited. After fertilization, the vector length and vector angle decreased, suggesting that fertilization can alleviate carbon and phosphorus limitations in the soil of subtropical grassland. These findings provide a theoretical basis for the improvement and management of subtropical cultivated grassland. Our results show that the appropriate application of organic fertilizers or biochar can contribute to the enhancement and restoration of subtropical artificial grassland, thereby alleviating carbon and phosphorus limitation in soil.

Key words: soil enzyme activity; soil enzyme stoichiometry; nutrient limitation; organic fertilizer; biochar

草地是地球上分布最广的生态系统之一,在碳循环、生物多样性、调节气候等多种生态系统服务中发挥重要作用^[1]。然而,由于气候变化和人类活动(如过度放牧)等因素导致草地退化,进而影响土壤肥力以及微生物群落结构和组成^[2]。土壤酶活性是评价土壤微生物功能和土壤养分循环的重要指标^[3]。土壤微生物通过其生长和新陈代谢活动,促进土壤有机物的分解与积累,并调节养分循环^[4],这些过程依赖于微生物的生物量和活性。微生物养分限制理论指出,微生物的生物量和活性往往受到碳(C)、氮(N)和磷(P)的限制^[5]。C、N和P获取酶的活性通常被归类为C循环相关的酶: β -葡萄糖苷酶(β -glucosidase, β G)、N循环相关的酶: β -1,4-N-乙酰葡萄糖苷酶(β -1,4-N-acetyl glucosidase, NAG)和亮氨酸氨基肽酶(leucine aminopeptidase, LAP)和P循环相关的酶:碱性磷酸酶(alkaline phosphatase, ACP)^[6–7]。土壤中酶活性以及酶化学计量比反映土壤养分代谢和微生物能量的情况^[8]。土壤微生物C、N和P的养分限制由土壤酶活矢量长度和矢量角度阐释^[9]。酶活矢量长度反映了微生物C限制大小,矢量长度越短表明C利用效率越高。酶活矢量角度表示N、P限制,酶活矢量角度大于 45° 表示受P限制,而小于 45° 表示受氮限制^[9]。Sinsabaugh等^[10]发现,全球土壤酶C:N:P的化学计量比约为1:1:1,由于环境和生物因素的差异,该比例略有偏差^[11]。

研究发现,不同植被类型、放牧强度、施肥类型等因素影响土壤酶活性和微生物群落特征^[12],其原因是土壤湿度、pH、养分及有机质含量等多种因素影响所致^[13]。Turner^[14]的研究表明,在酸性土壤中,NAG和LAP活性受到抑制,这与土壤酸性环境对微生物群落的抑制作用密切相关。水分也是影响土壤酶活性的重要因素之一,土壤水分的变化直接影响土壤微生物的生长和代谢活性,从而改变酶的活性^[14–16]。土壤有机碳和全氮是土壤微生物的重要碳源和氮源^[17],其含量的增加可以促进土壤微生物的生长和繁殖,进而提高土壤酶活性^[18]。

红池坝草地位于长江流域三峡库区腹地。作为三峡库区极佳的环境保护屏障,受到气候变化和人为干扰的影响而退化^[19]。草地退化导致土壤质量下降、养分流失和生态功能退化^[20]。在退化过程中,土壤酶活性是衡量土壤健康和微生物活性的重要指标,受多种非生物因素的影响^[21]。施肥是简单易行、见效快的改良措施^[22],通过补充土壤养分,施肥能够显著调节土壤酶活性,进而影响土壤养分循环和微生物代谢^[23]。前人研究表明,施加有机肥^[24]和生物炭^[25]是缓解微生物资源限制的有效手段。Li等^[26]研究发现,有机肥能提高土壤酶的活性。Tian等^[27]的研究表明,施加有机肥后可增加氮获取酶活性及碳磷和氮磷获取酶的比例。生物炭常通过减轻碳限制来控制土壤的微生物群落结构^[27],Wang等^[28]研究发现生物炭通过影响土壤性质和调控细菌群落促进旱地土壤碳、氮、磷获取酶活性。但是,迄今为止生物炭和有机肥对亚热带草地土壤酶活性及酶化学计量的影响未见报道。鉴于此,本研究以亚热带人工草地为对象,通过施加有机肥和生物炭,分析不同施肥类型下土壤理化性质、碳氮磷循环相关酶的活性及其化学计量特征,阐明施肥对亚热带人工草地土壤微生物养分限制的缓解效应,探讨施肥对土壤酶活性以及化学计量的驱动机制,为亚热带退化人工草地恢复治理和土壤改良提供科学依据。

1 材料与方法

1.1 研究区概况

研究区域位于重庆市巫溪县红池坝国家森林公园亚热带人工草地(108°51′-109°11′ E, 31°31′-31°40′ N, 海拔1800~2500 m),年平均气温为7.1℃,年降水量约为1953 mm,年蒸发量在1000~1200 mm,相对湿度为65%~88%,无霜期约为172 d,年平均日照时间约为1379 h。土壤类型为黄棕壤,土壤质地为沙质粉沙中壤和粘质沙中壤。该地区人工草地植物群落优势种为红三叶(*Trifolium pratense*)。

1.2 试验设计与样品采集

试验样地于2020年进行围封,2023年5月开展试验处理,人工施用有机肥(organic fertilizer, O)(购买于云阳县农高永利农业科技有限责任公司,总氮3.51%、总磷3.64%、总钾4.05%)和生物炭(biochar, B)(购买于宁夏平罗县锦晟活性炭有限公司,有机质68.1%、总磷0.86%、总钾2.63%)。采用随机区组设计(randomized block design),设立3个试验区组,小区面积10 m×9 m,共计9个小区(3个区组×3个处理)。对照(CK,不施肥)、添加有机肥(O,800 kg·hm⁻²·年⁻¹)和添加生物炭(B,2%,其百分比为土壤表层20 cm土层中生物炭重与土壤干重的百分比)。每个小区之间间隔2 m的缓冲带。每个小区周围的地面上打入一块0.5 m宽PVC板,表面露出8 cm,以避免不同处理之间的干扰。试验期间不进行除草和浇水。

2024年6月,在试验地点采集了土壤样本。每个小区随机选取5个点,使用土钻分别采集0~10 cm、10~20 cm、20~30 cm的土芯(直径50 mm),然后轻轻混合,形成复合样本。每个处理3个重复,所有小区共计27份土壤样品。剔除根系、凋落物和石头,并用2 mm的筛网过筛。一部分样本储存在4℃冰箱中,测定土壤酶活性。另一部分样本风干后测量土壤理化性质。

1.3 指标测定

采用环刀法测定土壤容重^[29],采用pH计(pHS-3E,中国上海)测定土壤pH,依据《土壤农化分析》^[30]测定总氮(total nitrogen, TN)、总磷(total phosphorus, TP)、铵态氮(soil ammonia nitrogen, NH₄⁺-N)、硝态氮(soil nitrate nitrogen, NO₃⁻-N)含量,采用重铬酸钾外加热法测定有机碳(soil organic carbon, SOC)含量^[31]。

根据Steinweg等^[32]的方法测定土壤胞外酶活性。参照Verchot等^[33]的方法确定反应底物。

1.4 数据分析

土壤胞外酶化学计量比以C、N、P获取酶的比值计算^[10,34]。

$$\text{Enzyme C:N} = \frac{\ln \beta G}{\ln (NAG + LAP)} \quad (1)$$

$$\text{Enzyme C:P} = \frac{\ln \beta G}{\ln ACP} \quad (2)$$

$$\text{Enzyme N:P} = \frac{\ln (NAG + LAP)}{\ln ACP} \quad (3)$$

式中:Enzyme C:N表示土壤酶碳氮比;Enzyme C:P表示土壤酶碳磷比;Enzyme N:P表示土壤酶氮磷比; βG 为 β -葡萄糖苷酶(β -glucosidase);NAG为 β -1,4-N-乙酰葡萄糖苷酶(β -1,4-N-acetyl glucosidase);LAP为亮氨酸氨基肽酶(leucine aminopeptidase);ACP为碱性磷酸酶(alkaline phosphatase)。

采用酶活性矢量分析[矢量长度(vector length, VL)和矢量角度(vector angle, VA)]评价微生物养分限制^[9]:

$$VL = \sqrt{X^2 + Y^2} \quad (4)$$

$$VA = \text{Degress}[\text{atan } 2(X, Y)] \quad (5)$$

式中:X为Enzyme C:P,Y为Enzyme C:N;VL表示微生物碳限制大小;VA>45°表示受磷限制,VA<45°表示受氮限制^[35]。

使用R 4.4.2(<https://www.r-project.org/>)进行单因素方差分析(ANOVA)并进行Tukey检验,采用Pearson进行相关性分析和逐步回归,利用R 4.4.2软件进行绘图。

2 结果与分析

2.1 施肥对亚热带人工草地土壤理化性质的影响

施肥后亚热带人工草地土壤理化性质存在差异(表 1)。O 和 B 处理增加了各土层含水量、pH、SOC、TN、TP、TK。O 和 B 处理显著降低了各土层土壤容重($P < 0.05$)。在 0~10 cm、20~30 cm 土层中,O 处理较 CK 显著增加 NH_4^+ -N 含量($P < 0.05$),B 处理显著增加 0~10 cm 土层 NH_4^+ -N 含量($P < 0.05$)。B 处理较 CK 显著增加各土层 NO_3^- -N 含量,在 0~10 cm、20~30 cm 土层中,O 处理显著降低了 NO_3^- -N 含量($P < 0.05$)。O 和 B 处理增加了各土层 C:N,但 0~10 cm 土层中 O 处理较 CK 差异不显著($P > 0.05$)。O 处理显著降低了各土层 C:P、N:P,B 处理显著降低了 0~10 cm、10~20 cm 土层的 N:P ($P < 0.05$)。

2.2 酶活性及其化学计量特征

O 处理显著增加各土层 βG 活性($P < 0.05$, 图 1),0~10 cm、10~20 cm、20~30 cm 各土层较 CK 分别提高了 36.18%、37.21%、59.30%,B 处理仅在 0~10 cm 土层 βG 活性较 CK 显著高 25.88% ($P < 0.05$),与施加生物炭相比,施加有机肥能增加更多的 βG 活性。但施加有机肥和生物炭的 LAP 活性与 βG 活性的响应相反,B 处理较 O 处理能增加更多的 LAP 活性($P < 0.05$)。O 和 B 处理增加各土层 NAG 活性,其中在 0~10 cm、10~20 cm、20~30 cm 土层中,O 处理较 CK 分别显著提高 NAG 活性 21.16%、17.25%、30.24% ($P < 0.05$),仅在 20~30 cm 土层中,B 处理较 CK 的 NAG 活性差异显著($P < 0.05$),与施加生物炭相比,施加有机肥能增加更多的 NAG 活性。在 20~30 cm 土层中,O 处理较 CK 显著提高 ACP 活性 8.82% ($P < 0.05$),在 0~10 cm、20~30 cm 土层中,B 处理较 CK 显著提高 ACP 活性 60.64%、25.96% ($P < 0.05$)。

O 和 B 处理在各土层的酶 C:N 均显著低于 CK ($P < 0.05$, 图 2),O 和 B 处理在各土层的酶 N:P 均显著高于 CK ($P < 0.05$),O 处理显著增加各土层酶 C:P ($P < 0.05$)。酶 C:P 和酶 N:P 小于 1,而酶 C:N 大于 1。C、N、P 获取酶的自然对数比

表 1 施肥对不同土层土壤理化性质的影响

Table 1 Effect of fertilizer application on soil physical and chemical properties of different soil layers

项目 Item	0~10 cm			10~20 cm			20~30 cm		
	CK	O	B	CK	O	B	CK	O	B
土壤含水量 Soil moisture content (%)	34.05±0.35c	35.52±0.09b	37.01±0.37a	28.50±0.30b	33.07±0.28a	32.85±0.24a	26.38±0.07c	34.77±0.20a	29.37±0.40b
土壤容重 Soil bulk density ($\text{g}\cdot\text{cm}^{-3}$)	1.46±0.01a	1.43±0.01b	1.42±0.02c	1.51±0.02a	1.50±0.03b	1.50±0.02b	1.52±0.03a	1.49±0.02c	1.50±0.03b
pH	4.88±0.01c	6.01±0.03a	5.06±0.02b	5.00±0.01b	5.74±0.01a	5.01±0.02b	4.93±0.06c	5.70±0.02a	5.13±0.04b
土壤铵态氮 Soil ammonia nitrogen ($\text{mg}\cdot\text{kg}^{-1}$)	5.19±0.01c	6.54±0.02a	5.50±0.01b	3.39±0.04a	3.20±0.05b	3.33±0.06ab	3.51±0.11b	4.70±0.03a	2.62±0.06c
土壤硝态氮 Soil nitric nitrogen ($\text{mg}\cdot\text{kg}^{-1}$)	8.77±0.08b	4.89±0.05c	11.34±0.06a	6.44±0.26b	7.76±0.12a	8.28±0.05a	5.54±0.07b	5.06±0.01c	5.88±0.01a
全氮 Total nitrogen ($\text{g}\cdot\text{kg}^{-1}$)	1.92±0.02c	2.11±0.01b	2.25±0.01a	1.65±0.02c	1.78±0.02b	1.91±0.02a	1.58±0.03b	1.61±0.01b	1.65±0.02a
全磷 Total phosphorus ($\text{g}\cdot\text{kg}^{-1}$)	0.63±0.01c	0.87±0.01a	0.79±0.01b	0.50±0.01c	0.60±0.03b	0.64±0.03a	0.48±0.03c	0.60±0.01a	0.52±0.01b
全钾 Total potassium ($\text{g}\cdot\text{kg}^{-1}$)	14.02±0.06c	14.78±0.04b	15.34±0.05a	14.55±0.01c	15.73±0.03a	15.58±0.02b	14.53±0.02c	14.98±0.04b	15.54±0.03a
碳氮比 Carbon to nitrogen ratio	9.05±0.04b	9.21±0.02b	11.22±0.17a	8.48±0.04c	9.24±0.12a	8.81±0.05b	7.95±0.05b	8.36±0.03a	8.39±0.08a
碳磷比 Carbon to phosphorus ratio	27.70±0.05b	22.36±0.01c	31.86±0.86a	27.85±0.07a	27.27±0.12b	26.17±0.09c	26.18±0.20a	22.40±0.22b	26.71±0.27a
氮磷比 Nitrogen to phosphorus ratio	3.06±0.01a	2.43±0.01c	2.84±0.03b	3.28±0.01a	2.95±0.03b	2.97±0.01b	3.29±0.01a	2.68±0.03b	3.18±0.06a
土壤有机碳 Soil organic carbon ($\text{g}\cdot\text{kg}^{-1}$)	17.41±0.05c	19.38±0.01b	25.27±0.46a	14.03±0.04c	16.42±0.19b	16.86±0.07a	12.55±0.08c	13.45±0.07b	13.83±0.05a

CK: 未施肥 No fertilizer; O: 施加有机肥 Organic fertilizer; B: 施加生物炭 Biochar; 不同小写字母表示在同一土层不同处理间差异显著 ($P < 0.05$); 下同 The same below.

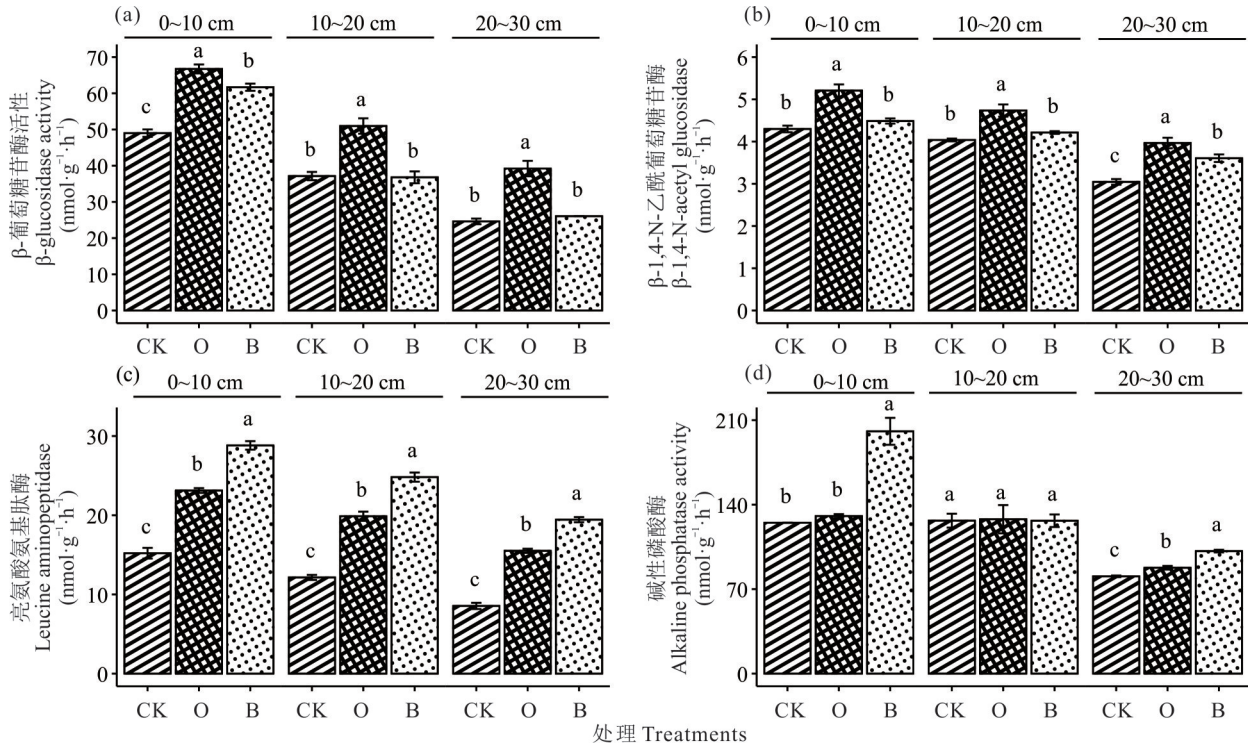


图1 不同施肥处理土壤酶活性变化

Fig. 1 Changes in soil enzyme activity under different fertilization treatments

不同小写字母表示同一土层不同处理间差异显著 ($P < 0.05$)。Different lowercase letters indicate significant differences in the same soil layer among different treatments ($P < 0.05$). CK: 未施肥 No fertilizer; O: 施加有机肥 Organic fertilizer; B: 施加生物炭 Biochar; 下同 The same below.

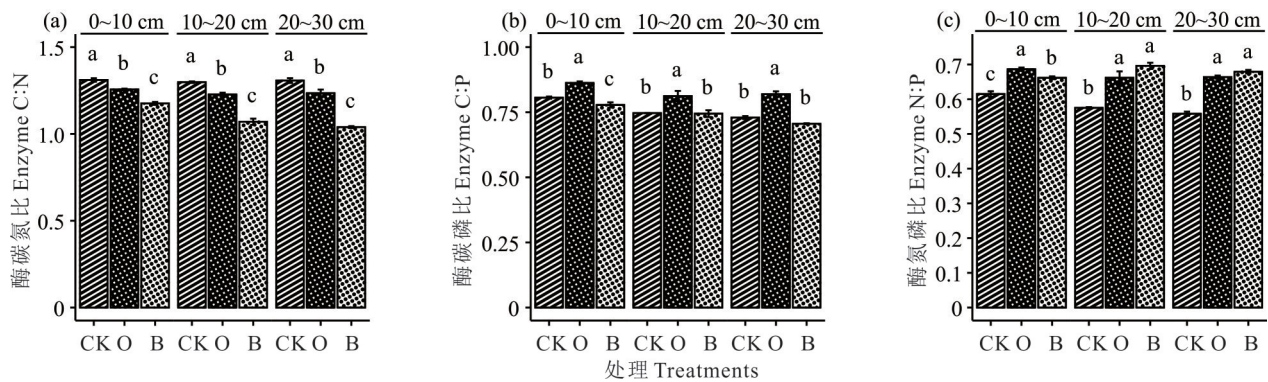


图2 不同施肥处理下土壤胞外酶化学计量比

Fig. 2 Soil extracellular enzyme stoichiometric ratios under fertilization treatments

值为 $1:1.04:1.47 \sim 1:1.31:1.79$, 偏离全球 $1:1:1$ 的平均值, 表明总体上存在 P 限制。O 和 B 处理后土壤酶的矢量分析(矢量长度和矢量角度)存在差异(图 3), 说明土壤微生物养分的供应受到施肥处理较大影响。B 处理显著降低各土层酶矢量长度 ($P < 0.05$), 说明亚热带人工草地土壤微生物碳磷代谢得到缓解。所有处理的酶矢量角度均大于 45° , 说明亚热带人工草地土壤微生物受到磷限制, 但施加 O 和 B 后, 各土层酶矢量角度较 CK 均显著降低 ($P < 0.05$), 表明施肥使微生物受磷限制得到缓解。养分限制特征内所有点均大于 $1:1$ 线(图 3c), 表明施加有机肥和生物炭, 可以降低微生物的磷限制。此外, 碳、氮、磷循环酶之间呈正相关(图 4)。

2.3 土壤酶活性及其化学计量与土壤理化性质之间的关系

βG 活性与 SM 、 $\text{NH}_4^+\text{-N}$ 、 TN 、 TP 、 SOC 极显著正相关 ($P < 0.01$, 图 5), 与 C:N 显著正相关 ($P < 0.05$)。NAG 活性与 TP 极显著正相关 ($P < 0.01$), 与 BD 、 N:P 显著负相关 ($P < 0.05$)。LAP 活性与 TN 、 SOC 极显著正相关

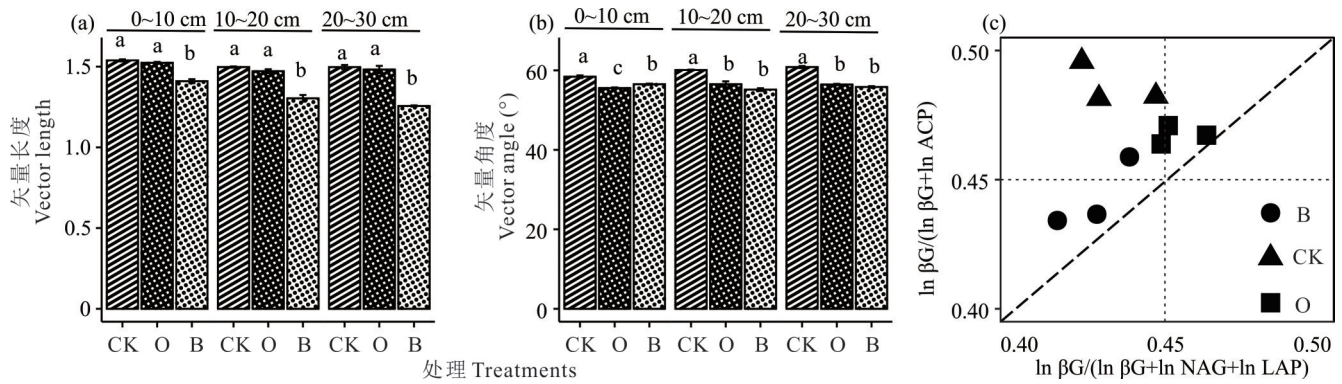


图 3 酶化学计量特征与土壤碳氮和碳磷获取酶化学计量比的相对比例

Fig. 3 Enzyme stoichiometric characteristics and the reative ratio of soil carbon-nitrogen and carbon-phosphorus acquiring enzyme stoichiometries

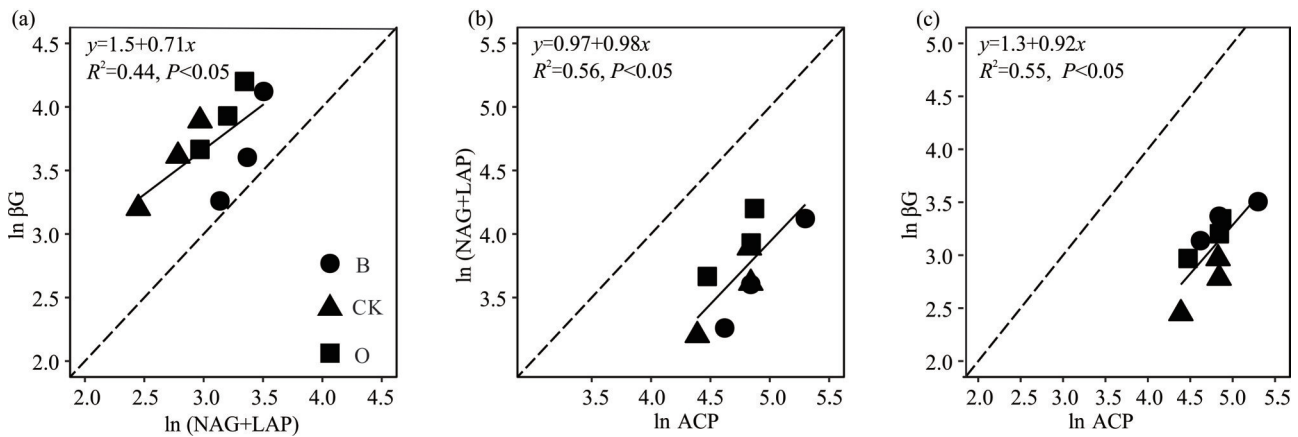


图 4 碳氮磷相关酶活性的标准主轴回归

Fig. 4 Standard major axial regression of enzyme activities related to carbon, nitrogen, and phosphorus

黑色虚线表示斜率为 1.0 的参考线,黑色实线表示线性拟合方程。The black dotted line represents a reference lines with a slope of 1.0, and the solid black line represent the linear fitting equation.

($P < 0.01$), 与 SM、TP、C:N 显著正相关 ($P < 0.05$)。ACP 与 NO_3^- -N、TN、C:N、SOC 极显著正相关 ($P < 0.01$), 与 BD 显著负相关 ($P < 0.05$)。酶 C:N 与 TK 显著负相关 ($P < 0.05$)。酶 C:P 与 SM、pH、 NH_4^+ -N、TP 极显著正相关 ($P < 0.01$), 与 N:P 极显著负相关 ($P < 0.05$)。酶 N:P 与 TK 显著正相关 ($P < 0.05$), 与 N:P 显著负相关 ($P < 0.05$)。VL 和 VA 均与 TK 呈显著负相关 ($P < 0.05$)。VA 与 N:P 显著正相关 ($P < 0.05$)。综上, SM、pH、 NH_4^+ -N、TP、TK、N:P 显著影响酶化学计量比。

通过冗余分析表明(图 6), 土壤理化性质对土壤酶活性的总解释率为 85.40%, 其中 RDA1 和 RDA2 的解释率分别为 71.20% 和 14.20%。土壤理化性质对土壤酶化学计量比的总解释率为 100%, 其中 RDA1 和 RDA2 的解释率分别为 57.48% 和 42.52%。

3 讨论

3.1 施肥对亚热带人工草地土壤理化性质的影响

本研究表明, 施加有机肥和生物炭可以提高亚热带人工草地土壤含水量、pH, 其原因可能是有机肥增强土壤团粒结构的同时, 改善土壤透气性, 从而提高土壤含水量^[36], 有机肥中含有的碱性物质将土壤中的酸性物质中和, 从而提高土壤 pH^[37]。较高的 pH 有利于铵态氮的稳定性, 有机肥促进微生物活动, 从而促进铵态氮的转化^[38], 但由于有机肥中的高有机物含量, 可能会促进一些抑制硝化作用的微生物的生长, 从而降低硝化作用的速率, 减少

硝态氮的生成^[39]。生物炭具有较大的比表面积和良好的孔隙结构^[40],从而减少水分的蒸发和流失,改善土壤团粒结构的同时增加孔隙度,使其具有更好的水气交换性,从而进一步增加土壤含水量。生物炭会中和土壤酸性成分,降低土壤酸性^[41]。生物炭还促进了硝态氮的积累,生物炭的碱性环境和高孔隙度可能刺激硝化细菌的活性,促使土壤中的铵态氮转化为硝态氮,从而提高了土壤中的硝态氮含量^[42]。Li等^[43]通过施用生物炭修复退化草地的试验发现,施用生物炭可增加土壤含水量、全氮、有机碳含量,本研究结果与此类似。本研究还发现,施加生物炭比施加有机肥会增加更多的SOC、TN含量,这与前人研究的结论一致^[44]。这可能是因为生物炭的高碳含量,具有较为坚固的分子结构和较低的生物降解速率^[45],生物炭具有高度稳定性和较强的吸附性能,通过吸附土壤中的有机物质和养分,减少其流失^[46],相比之下,亚热带人工草地高温高湿环境加速了有机肥的分解,使其对SOC的贡献进一步受限^[47],而生物炭由于其耐分解性,能够更稳定地提高土壤碳储量。另外,有研究发现生物炭可以增加土壤中与碳循环和氮循环相关的微生物的相对丰度,对土壤微生物群落的调控作用间接促进了SOC和TN的积累^[48]。

3.2 施肥对亚热带人工草地土壤酶活性及酶化学计量比的影响

土壤酶是土壤中的催化剂,参与土壤的物质循环和能量流动^[49],是反映土壤质量以及土壤中物质和能量代谢程度的重要生物指标^[50]。Dominchin等^[51]和Zhou等^[52]研究发现生物炭和有机肥可提高土壤中碳

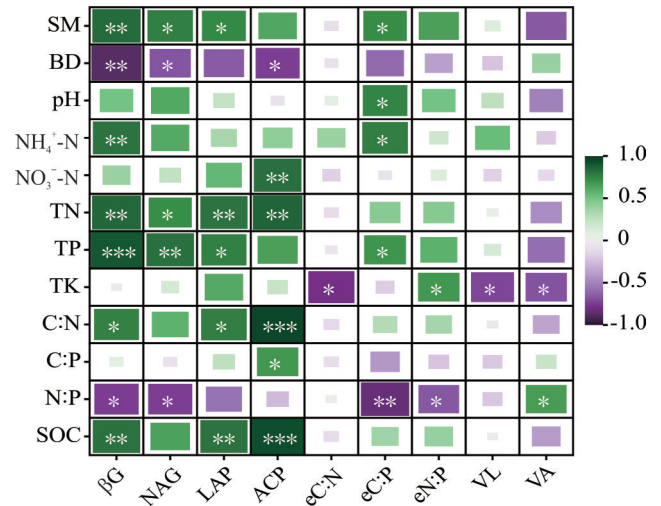


图5 土壤酶活性及其化学计量特征与土壤理化性质的相关性
Fig. 5 Correlation of soil enzyme activities and their stoichiometric characteristics with soil physicochemical properties

SM: 土壤含水量 Soil moisture; BD: 土壤容重 Soil bulk density; pH: 土壤pH; NH₄⁺-N: 土壤铵态氮 Soil ammonia nitrogen; NO₃⁻-N: 土壤硝态氮 Soil nitric nitrogen; TN: 土壤全氮 Soil total nitrogen; TP: 土壤全磷 Soil total phosphorus; TK: 土壤全钾 Soil total potassium; C:N: 土壤碳氮比 Soil carbon nitrogen ratio; C:P: 土壤碳磷比 Soil carbon phosphorus ratio; N:P: 土壤氮磷比 Soil nitrogen phosphorus ratio; SOC: 土壤有机碳 Soil organic carbon; β G: β -葡萄糖苷酶活性 β -glucosidase activity; NAG: β -1,4-N-乙酰葡萄糖苷酶 β -1,4-N-acetylglucosidase; LAP: 亮氨酸氨基肽酶 Leucine aminopeptidase; ACP: 碱性磷酸酶 Alkaline phosphatase activity; eC:N: 酶碳氮比 Enzyme C:N; eC:P: 酶碳磷比 Enzyme C:P; eN:P: 酶氮磷比 Enzyme N:P; VL: 矢量长度 Vector length; VA: 矢量角度 Vector angle; 下同 The same below; 方框中的绿色和紫色分别代表两个变量之间的正相关和负相关 Green and violet in the square represent positive and negative correlations between the two variables, respectively. 颜色越深,关系越强 The deeper the color, the stronger the relationship. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

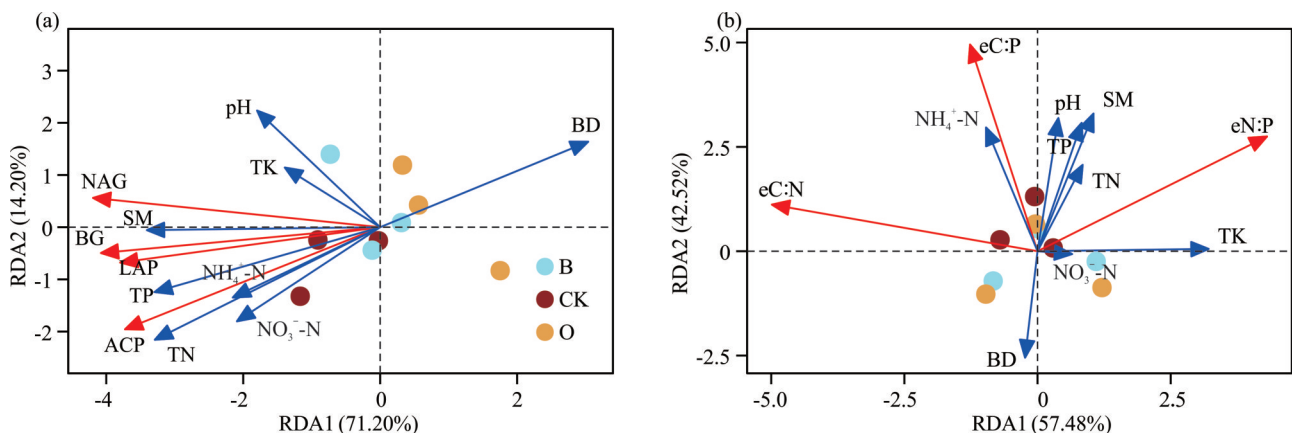


图6 土壤理化性质与 (a) 土壤酶活性以及 (b) 酶化学计量比的冗余分析

Fig. 6 Redundancy analysis of soil enzyme activity, stoichiometry, and physicochemical properties

的储存量和生物利用率,从而促进微生物的生长和与碳降解相关酶的合成。本研究施加有机肥和生物炭可以提高 β G 和 NAG 活性的结果与此一致(图 1a,b)。其中。施加有机肥比施加生物炭提高 β G 和 NAG 活性的幅度大,这可能是因为有机肥中富含可供微生物分解的有机碳源^[53],可使分解复杂碳水化合物方面具有生理优势的真菌类群活性增强^[54]。相比之下,生物炭虽然富含碳,但其结构较为稳定,导致微生物难以直接利用^[55]。这种碳源的差异,使得有机肥处理下的微生物更倾向于快速代谢策略(r-策略)^[56],从而显著增加 β G 和 NAG 活性。研究发现,土壤 LAP 和 ACP 活性在施加有机肥和生物炭后呈上升趋势,这是由于施加生物炭后,增强了土壤氮、磷矿化的微生物群落的活性^[57]。此外,生物炭稳定的碳源改善了土壤微生物的生境,增强了氮、磷矿化微生物的活性^[58],也与土壤中的养分发生相互作用,促进磷和氮的释放^[59]。此时,生物炭的施用通过提供更稳定的有机碳和改善土壤微环境的方式,促进了与氮、磷矿化相关的微生物群落的活性^[60],从而使得施加生物炭的 LAP 和 ACP 活性增幅更大(图 1c,d)。

3.3 施肥对亚热带人工草地土壤微生物养分限制的影响

本研究的 eC:N 小于全球尺度的平均值 1.41, eC:P、eN:P 高于全球尺度的平均值 0.62 和 0.44^[61],表示土壤养分处于氮限制和磷限制状态。在全球范围内,土壤碳氮磷获取酶的比例为 1:1:1,这表明微生物在不同生态系统中维持 C、N 和 P 的平衡有一个广泛的模式^[61],这一比例因生态系统类型和环境的不同而变化。本研究中,施加有机肥后的碳氮磷获取酶的平均比例为 1:1.24:1.49,施加生物炭后的碳氮磷获取酶的平均比例为 1:1.10:1.47,偏离了 1:1:1,表明 C 获取酶活性相对小于 N 和 P 获取酶活性,P 获取酶活性相对大于 C 和 N 获取酶活性,微生物存在氮限制和磷限制。根据标准主轴回归分析中的斜率参数,其偏离 1 的程度可用于定量表征土壤养分的相对限制程度^[62]。本研究结果显示,ln β G 与 ln AP 的回归斜率为 0.97,与 1 偏离程度大,这意味着土壤微生物群落的磷限制最为显著。磷是植物和微生物生长的限制性元素之一,而较高的 eC:P 和 eN:P 通常表明土壤微生物对磷的需求较为强烈,这可能是由于土壤中磷的有效性较低^[63]。磷在许多亚热带土壤中常常因与铁、铝等金属形成难溶性化合物而处于不可利用状态^[64]。尽管有机肥和生物炭的施用改善了土壤的碳和氮供应,但由于磷的生物可利用性较低,微生物和植物仍然受限于磷的供应,从而导致 eC:P 和 eN:P 上升,指示土壤处于磷限制状态。土壤酶化学矢量长度、矢量夹角是评估微生物养分限制的关键指标^[65]。矢量长度表示微生物受到碳限制,矢量夹角大于 45° 表明微生物受磷限制^[9]。本研究施肥后矢量长度、矢量夹角较 CK 降低,说明施肥可以缓解碳、磷限制。这与 Cui 等^[66]的研究结论一致,即有机肥处理降低矢量长度、矢量夹角,缓解碳磷代谢失衡。有机肥促进微生物群落的生长与繁殖,增加土壤中的磷酸酶活性,从而加速了土壤中有机磷和无机磷的转化过程^[67]。磷酸酶能将土壤中的有机磷转化为可被植物和微生物吸收的无机磷,有效地缓解了磷的限制^[68]。有机肥改善土壤的物理化学性质,减少磷与土壤中的铝、铁、钙等元素的结合,降低磷的固定程度^[68]。磷不容易被固定时,微生物和植物能够更容易地利用土壤中的磷,从而缓解磷限制。生物炭的应用减小了矢量长度、矢量夹角并减轻了微生物碳、磷限制^[69],因为生物炭给微生物提供额外的碳源^[70],以及生物炭的表面形成了相对稳定的土壤有机碳(SOC)复合物,在一定程度上保护了 SOC 的快速分解,使其能够继续缓慢释放碳源^[71]。此外,生物炭促进了微生物群落组成,能够促进磷的矿化与转化、利用碳等营养物质,并提高了碳的周转率以及磷的可用性^[72]。

土壤 pH、容重、全氮等理化性质会影响土壤酶活性^[72-74]。本研究表明, β G 活性与 SM、 NH_4^+ -N、TN、TP、SOC、C:N 显著正相关,这与前人研究一致^[75],主要是因为含水量的增加可以增强微生物活性^[76],优化土壤结构^[77],导致土壤 β G 活性增加。Singh 等^[78]研究表明,SOC 与 β G 活性呈正相关,SOC 为微生物提供底物和碳源促进 β G 活性以及黄海莉等^[79]研究发现 β G 活性与 NH_4^+ -N、TN、SOC 正相关, NH_4^+ -N 和 TN 作为氮源,促进微生物生长和酶的合成,进一步提高 β G 活性^[80]。SOC 和 TN 为提供丰富的酶作用底物^[34],使得 SOC 和 TN 与 LAP 和 ACP 的活性呈正相关。

4 结论

本研究发现,施加有机肥和生物炭显著增加亚热带人工草地土壤 SOC、TN 含量,增加土壤 pH。施加有机肥

显著增加各土层 β G、NAG活性,施加生物炭显著增加各土层LAP、NAG活性。土壤TN是显著影响土壤酶活性的重要因子。亚热带人工草地土壤微生物受到碳和磷限制,施肥能缓解微生物碳和磷限制,其中与有机肥相比施加生物炭能缓解更多的碳限制。本研究结果可为亚热带人工草地改良或退化人工草地恢复提供科学依据。

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