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萘对川西亚高山森林土壤呼吸、养分和酶活性的影响

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摘 要 萘作为土壤动物化学抑制剂已在土壤动物生态功能的研究中广泛使用,但其非目标效应使其应用仍存在很大的不确定性.为了了解在亚高山森林土壤应用萘抑制土壤动物群落是否存在非目标效应,以青藏高原东缘的川西亚高山森林土壤为研究对象,采用微缩试验研究了萘对土壤呼吸速率、养分含量和酶活性的短期影响.结果表明: 萘处理显著抑制了培养0~10 d的土壤呼吸速率,随后(24~52 d)表现出明显的促进作用.萘处理显著影响了土壤铵态氮和硝态氮含量的动态变化,萘处理铵态氮和硝态氮含量分别以培养的3和17 d最高,对照则以培养的45 d和结束时的52 d最高.萘处理土壤可溶性碳含量在培养3 d急剧增加后迅速降低,对照则略有升高后降低,而萘处理和对照的可溶性氮含量均表现为先升高后降低.萘处理和对照的土壤酶活性均具相似的动态规律,两者的脲酶、硝酸还原酶和亚硝酸还原酶活性分别在培养45、38和10 d至最高.萘处理和采样时间的交互作用显著影响了土壤呼吸速率,以及土壤铵态氮、硝态氮和可溶性氮含量,但对可溶性碳含量、蔗糖酶、硝酸还原酶和亚硝酸还原酶活性的影响不显著.萘作为驱虫剂的非目标效应可能在短期内对川西亚高山森林土壤的氮循环过程产生强烈的影响.

关键词 萘; 土壤呼吸; 土壤养分; 土壤酶活性; 亚高山森林

Effects of naphthalene on soil respiration, nutrients and enzyme activities in the subalpine forest of western Sichuan, China.

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Abstract: As a biocide to reduce soil and litter faunal populations in field experiments, naphthalene has been widely used in the study of ecological functions of soil fauna, but the non-target effects of naphthalene bring about enormous uncertainty to its application. In order to understand whether there were non-target effects of naphthalene in subalpine forest soil, soil in the subalpine forests of west Qinghai-Tibet Plateau was taken as study object. The short-term responses of soil respiration rate, nutrient content and enzyme activity to naphthalene were studied in microcosms. The results showed that soil respiration rate was significantly suppressed by application of naphthalene within 0-10 days, and then showed a significant promotion effect. Naphthalene significantly affected

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the dynamics of soil NH_4^+-N and NO_3^--N contents. With application of naphthalene, the highest contents of NH_4^+-N and NO_3^--N occurred at the 3rd and 7th day, respectively. But they were observed at the 45th and 52nd day with no-naphthalene, respectively. Moreover, soil dissolved carbon content in the naphthalene microcosms showed a sharp increase and then decrease dynamic at the 3rd day, while small change was detected in the no-naphthalene microcosms. Dissolved nitrogen content in both the naphthalene and no-naphthalene microcosms showed an increase at first and then decreased subsequently during the study period. Similar dynamics were found for the soil enzyme activities in both the naphthalene and no-naphthalene microcosms. The highest activities of urease, nitrate reductase and nitrite reductase in both the naphthalene and no-naphthalene microcosms were at the 45th, 38th and 10th day, respectively. In addition, the interaction of naphthalene treatment and sampling time had significant effects on soil respiration rate, the contents of NH_4^+-N, NO_3^--N and dissolved nitrogen, but had no significant effects on soil dissolved carbon content, and the activities of invertase, nitrate reductase and nitrite reductase. In a short time, the non-target effect of naphthalene as a biocide to reduce soil fauna abundance might have an important influence on the soil nitrogen cycling in subalpine forest of western Sichuan.

Key words: naphthalene; soil respiration; soil nutrient; soil enzyme activity; subalpine forest.

土壤动物与微生物形成的碎屑食物链决定着森林地表的物质循环和能量转换[1].有研究表明,土壤动物一方面可通过吞噬、破碎、混合等直接作用,增加凋落物自身与土壤微生物群落的接触表面积,进而加速凋落物养分流动速率[2-3];另一方面可通过对微生物的选择性取食、繁殖体的接种传播,以及微生物群落结构的改变,间接作用于凋落物分解速率,进而影响土壤物质循环的速率和方向,以及整个土壤养分库营养效率[4-5].目前,基于分解袋法原理,采用物理排除或化学抑制控制土壤动物群的参与是研究其对凋落物分解影响的主要手段[3,6].萘作为土壤动物化学抑制剂已在探讨土壤动物生态功能的研究中广泛使用,然而,抑制剂的非目标效应在控制土壤动物活动的同时也可能极大地影响了土壤微生物活性和养分循环过程[7-8],且抑制效率和非目标效应也随土壤、植被类型、凋落物基质质量和水热环境的不同存在显著差异.例如,Blair 等[8]研究发现,萘显著降低了亚热带森林土壤和凋落物中有效氮含量和真菌数量;Cotrufo 等[9]研究表明,萘在有效抑制温带森林土壤节肢动物的同时,并未对土壤线虫、微生物生物量和土壤碳动态造成显著影响;Seastedt[10]则指出,萘抑制效率在热带森林和湿润土壤中比温带森林更明显.由此可见,已有的研究结论应用在某些特定生态系统中可能还具有很大的不确定性,如受季节性冻融和雪被显著影响的亚高山/高山森林生态系统,但相关研究未见报道.为此,本文以青藏高原东缘的川西亚高山森林土壤为研究对象,采用微缩试验研究了土壤呼吸、有效氮养分和氮转化酶活性对萘胁迫的短期响应,以期为在亚高山森林生态系统采用萘作为土壤动物群落抑制剂,研究土壤动物生态功能及其与物质循环的关系提供相关参考.

1 研究地区与研究方法

1.1 研究区概况

供试土壤采自四川省毕棚沟自然保护区高山森林生态系统定位实验研究站次生林群落(31°18′ N, 102°56′ E,海拔 3023 m).该群落乔木以岷江冷杉(*Abies faxoniana*)为主,树龄 80 年,郁闭度 0.7,林下灌木主要有:箭竹(*Fargesia spathacea*)、三颗针(*Berberis julianae*)、红毛花楸(*Sorbus rufopilosa*)、沙棘(*Hippophae rhamnoides*)、扁刺蔷薇(*Rosa weginzowii*)等,草本植物主要有:蟹甲草(*Cacalia auriculata*)、冷蕨(*Cystopteris montana*),以及苔草属(*Carex* spp.)和莎草属(*Cyperus* spp.)植物等[11].森林土壤为湿润雏形土,土壤有机层厚度(12.8±1.7) cm,pH 值(6.5±0.3),土壤全碳、全氮和全磷含量分别为(153.9±27.4) g · kg^{-1}、(7.8±1.3) g · kg^{-1}、(0.9±0.1) g · kg^{-1}[11-12].

1.2 试验设计

2015 年 10 月下旬采集供试土壤.在已建立的岷江冷杉次生林群落 1 hm^2 正方形固定样地中,随机选择 3 个 5 m×5 m 样方,在每个样方中采集 0~15 cm 土层混合土壤,共 10 kg.将样品装入冰盒低温处理,24 h 内运回实验室,将样品去掉石块、动植物残体、根系和可见的大中型土壤动物后,混匀,过 2 mm 筛.参照 Blair 等[8]方法进一步去除微小型土壤动物和线虫,处理周期为 24 h.

2015 年 11 月初进行培养试验.称取 50 g 土壤样品装入 450 mL 组织培养瓶,共 80 个.随机选择 40 个组织培养瓶施用萘作为处理组(8 次采样×5 个重复),施用量每瓶 1 g[9],每月添加 1 次;剩余 40 个组织培养瓶不施用萘,作为对照.将组织培养瓶用凡士林密封置于人工气候箱培养待测.同时,准备 10 个无土组织培养瓶(5 个处理+5 个对照)用以检验密封效果.培养时间为 2 个月,基于前期土壤温度和含水量监测数据,培养温度为 10 ℃,采用差量法[13]控制土壤含水量(45%).

1.3　样品采集与试验分析

于处理后的第 3、10、17、24、31、38、45 和 52 天采集土壤样品,每次采集处理和对照各 5 瓶,去除杂质后用于土壤养分和酶活性测定.采用碱液吸收法测定土壤 CO_2 排放,计算土壤呼吸速率,每周更换 1 次碱液[14].铵态氮(NH_4^+-N)采用靛酚蓝比色法测定;硝态氮(NO_3^--N)采用酚二磺酸比色法测定[15].同时,采用 0.5 mol · L^{-1} K_2SO_4 浸提土壤中可溶性碳和可溶性氮:称取 3 份 10 g 土壤样品于 150 mL 提取瓶中,加入 50 mL 0.5 mol · L^{-1} K_2SO_4 浸提液,振荡浸提 30 min,用定量滤纸过滤,再用 0.45 μm 滤膜抽滤,滤液采用总有机碳分析仪(TOC-VcPH+TNM-1, Shimazu Inc., Kyoto, Japan)测定[16].土壤蔗糖酶和脲酶活性参考关松荫[15]的方法;土壤反硝化酶(硝酸还原酶、亚硝酸还原酶)活性测定采用武志杰等[17-18]的方法.土壤蔗糖酶活性采用 3,5-二硝基水杨酸比色法测定,一个酶活性单位以 1 g 土壤在 37 ℃条件下 24 h 内各自水解产生的葡萄糖毫克数表示.土壤脲酶活性采用脲素比色法测定,一个酶活单位以 1 g 土壤在 37 ℃下 24 h 内反应水解产生的 NH_4^+ 毫克数表示.土壤硝酸还原酶和亚硝酸还原酶活性用苯磺酸-醋酸-α-萘胺比色法测定,其中,硝酸还原酶一个酶活单位以 1 kg 土壤在 30 ℃下 24 h 内还原产生的 NO_2^- 的毫克数表示;亚硝酸还原酶一个酶活单位以 1 kg 土壤在 30 ℃下 24 h 内还原减少的 NO_2^- 的毫克数表示.

1.4　数据处理

采用 SPSS 20.0 软件进行数据统计分析.利用重复测量方差分析(repeated measures ANOVA)检验萘处理和采样时间及二者交互作用对土壤酶活性的影响,采用单因素方差分析(one-way ANOVA)和最小显著差异法(LSD)检验各变量在不同处理或采样时间的差异显著性($\alpha = 0.05$).利用 SigmaPlot 12.5 软件作图.

2　结果与分析

2.1　萘对土壤呼吸速率的影响

在整个培养期间,萘处理和对照的土壤呼吸速率大体上呈现降低的趋势(图 1).萘处理土壤呼吸速率以培养 17 d 最高,对照则以培养 3 d 最高,二者均以培养结束时(52 d)最低.萘处理显著抑制了培养0~10 d的土壤呼吸速率,随后(24~52 d)表现出明显的促进作用.萘处理、采样时间及二者交互作用显著影响了土壤呼吸速率(表 1).

2.2　萘处理对土壤碳和养分含量的影响

在整个培养期间,萘处理显著影响土壤碳和养分含量的动态变化(图 2).萘处理铵态氮含量表现为先降低后升高再降低的动态,以培养初期(3 d)最高,结束(52 d)时最低;对照则为先降低后升高的动态,以培养 45 d 时最高,10 d 时最低.萘处理硝态氮含量表现出先升高后降低的动态,以培养 17 d 时最高,培养 45 d 时最低;对照则为升高的动态,以培养结束最高,初期最低.萘处理土壤可溶性碳含量在培养初期急剧增加后降低,对照则先略有升高后降低,以培养 10 d 时最高.萘处理和对照可溶性氮含量均表现为先升高后降低的动态,萘处理以培养 17 d 时最高,结束最低,对照则以培养 45 d 时最高,初期最低.萘处理和采样时间的交互作用显著影响了铵态氮、硝态氮和可溶性氮的含量,但对可溶性碳含量影响不显著(表 1).

2.3　萘对土壤酶活性的影响

整个培养期间,萘处理和对照土壤酶活性均具有相似的动态变化(图 3).蔗糖酶活性总体呈降低的趋势,萘处理在培养 17 d 时下降至活性最低值,对照在培养31 d时下降至活性最低值;脲酶活性

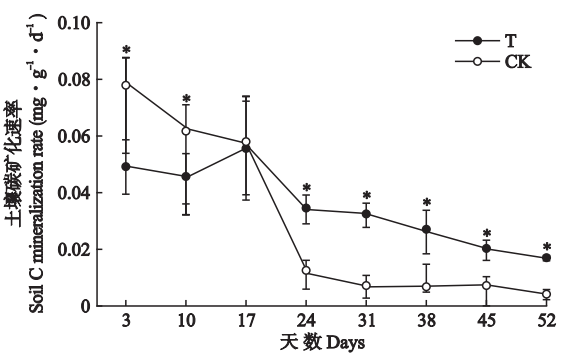

图 1　萘处理对川西亚高山森林土壤呼吸速率动态的影响

Fig.1　Effects of naphthalene on dynamics of soil respiration rate in the subalpine forests of western Sichuan.

* $P<0.05$. T: 萘处理 Naphthalene treatment; CK: 对照 Control. 下同 The same below.

表 1 萘处理和采样时间对川西亚高山森林土壤呼吸速率、养分含量和酶活性的重复测量方差分析

Table 1 Repeated measures ANOVA for soil respiration rate, nutrient content and enzyme activities to naphthalene treatment and sampling time

	土壤呼吸速率 Soil respiration rate	铵态氮 NH_4^+-N	硝态氮 NO_3^--N	可溶性碳 Dissolved carbon	可溶性氮 Dissolved nitrogen	蔗糖酶 Invertase	脲酶 Urease	硝酸还原酶 Nitrate reductase	亚硝酸还原酶 Nitrite reductase
T	30.45**	445.28**	62.06**	0.06	68.46**	2.11	22.28*	0.30	1.63
D	303.20**	26.62**	11.37**	101.87**	7.21**	7.98**	240.05**	35.90**	26.30**
T×D	62.86**	56.80**	18.97**	74.45**	6.59**	1.12	5.63*	1.02	0.75

* $P<0.05$; ** $P<0.01$. T: 萘处理 Naphthalene treatment; D: 采样时间 Sampling date.

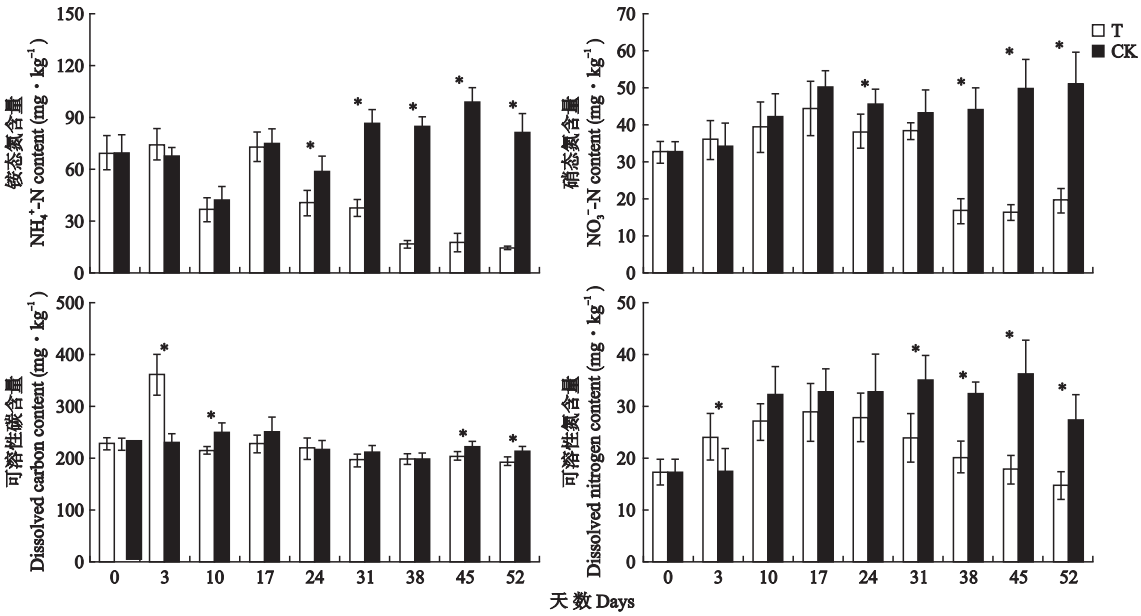

图 2 萘对川西亚高山森林土壤铵态氮、硝态氮、可溶性碳和可溶性氮含量动态的影响

Fig.2 Effects of naphthalene on dynamics of soil NH_4^+-N, NO_3^--N, dissolved carbon and dissolved nitrogen content in the subalpine forests of western Sichuan.

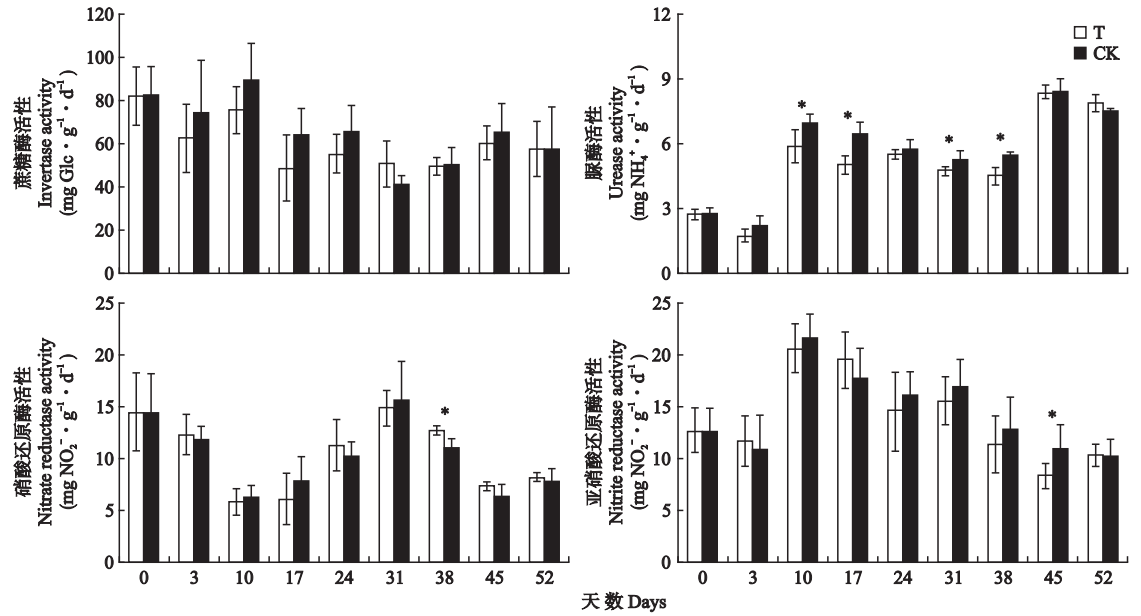

图 3 萘对川西亚高山森林土壤蔗糖酶、脲酶、硝酸还原酶和亚硝酸还原酶活性的影响

Fig.3 Effects of naphthalene on activities of soil invertase, urease, nitrate reductase and nitrite reductase in the subalpine forests of western Sichuan.

表现为先降低后升高的变化趋势,萘处理和对照均在培养初期下降至活性最低值,45 d 时上升到活性最高值;硝酸还原酶活性表现为先降低后升高再降低的变化趋势,萘处理和对照均在培养 10 d 时下降至活性最低值,38 d 时升高到活性最高值;亚硝酸还原酶活性表现为先升高后降低的变化趋势,萘处理和对照在培养 10 d 时升高至活性最高值,但萘处理亚硝酸还原酶活性在培养 45 d 时降低至活性最低值,对照则在培养结束降低至活性最低值.萘处理并未显著影响整个培养期间土壤蔗糖酶活性,但显著抑制了土壤脲酶活性,采样时间显著影响了土壤蔗糖酶、硝酸还原酶和亚硝酸还原酶活性,萘处理和采样时间显著影响了土壤脲酶活性,但二者交互作用不显著(表 1).

3　讨　　论

土壤可溶性有机质是土壤中易分解的碳源和养分资源,能够被微生物群落快速矿化为 CO_2,并被植被直接利用的无机氮素养分(铵态氮和硝态氮),对环境干扰和水热动态变化的响应敏感[19].早期模拟试验指出[20-21],萘添加到土壤后可能被土壤有机质吸附并作为碳源基质供给微生物群落利用,进而影响土壤呼吸速率和碳循环过程.在微缩试验中,土壤微生物异氧呼吸是 CO_2 排放的主要来源,环境干扰(如重金属污染、持久性有机污染物、土地利用)初期常常能对微生物生物异氧呼吸产生显著的激发效应[7-8,22].与 Blair 等[8] 研究结果相似,本研究中,萘处理早期并未明显提高土壤呼吸速率,相反,显著抑制了培养初期(0~10 d)的土壤呼吸速率.这是由于部分耐受性低的微生物群落受萘处理影响短期内大量死亡(图 1),微生物死亡释放的碳和养分一方面可促进土壤可溶性碳和可溶性氮在土壤中的迅速累积(图 2)[21],另一方面这些释放的有效基质可被存活的土壤生物直接利用,或促进萘处理环境中土壤微生物群落结构的快速调整,短期提高呼吸速率[20].这与相关的持久性有机污染物研究结果一致[8,20,23].然而,这种短期的激发效应会随着土壤有效基质的消耗和微生物群落的结构稳定迅速消失,土壤呼吸速率和养分含量逐渐回落并趋于稳定.同时,Cotrufo 等[9] 采用同位素碳示踪的研究显示,$\delta^{13}C$ 标记的萘并未被土壤微生物群落转化或作为碳源用作呼吸代谢,因此,培养后期萘处理环境下土壤呼吸速率显著高于对照,更可能是环境干扰持续存在造成土壤微生物活性不断变化的结果.相比于土壤呼吸速率和可溶性碳含量的变化,土壤可溶性氮和有效氮含量在培养后期受萘处理影响显著降低.由于缺乏地上植被根系吸收利用,这可能是萘处理干扰下微生物群落结构(如丰度、生物量)和养分利用效率与对照存在明显差异的原因[8].可见,萘作为驱虫剂的非目标效应可能在短期内对川西亚高山森林土壤的氮循环过程产生强烈的影响.

土壤酶活性能有效反映土壤生物化学代谢强度,受到土壤水热条件、植被类型和环境干扰调控[23-24].目前,关于多环芳烃(PAHs)有机物污染的研究表明,土壤酶活性通常在低浓度污染环境中表现出促进作用,在高浓度污染环境中则为强烈的抑制作用[25-26].例如,菲污染农田的治理过程中,在修复前期土壤蔗糖酶活性逐渐升高,在第 14 天达到峰值后逐渐下降并趋于稳定,而且在 50 mg · kg^{-1} 菲污染样品中土壤蔗糖酶活性高于 5 mg · kg^{-1} 的样品[23].在 PAHs 长期污染的水稻田 0~20 cm 表层土壤中,PAHs 浓度为 0~1.2 mg · kg^{-1},PAHs 浓度越高,脲酶活性越高;但随着 PAHs 浓度的升高,脲酶活性的抑制作用愈加明显[25].微缩试验研究认为,萘处理对真菌群落生长、繁殖和相关生物酶合成的抑制是导致土壤活性出现降低的主要原因[8];而原位培养试验发现,萘处理并未影响真菌和细菌的总磷酸脂肪(PLFA)丰度,仅对革兰氏阴性细菌 PLFA 丰度造成显著影响,因而萘并不会对土壤微生物群落结构和活性产生影响[9].本研究中,尽管萘对土壤酶活性产生一定的抑制效应,蔗糖酶、硝酸还原酶和亚硝酸还原酶活性并未受到萘处理的显著影响(表 1 和图 3).值得注意的是,作为氮素转化环节上的 3 种相互联系酶,即脲酶、硝酸还原酶和亚硝酸还原酶活性均随土壤可溶性氮和有效氮含量变化表现出明显的动态过程,且萘处理显著影响了脲酶活性.脲酶具有专化作用,影响其活性的因素主要分为底物数量和能够与酶结合阻止催化作用的抑制物[27].与对照相比,萘处理导致培养过程中土壤可溶性氮和有效氮含量的降低(图 2),可能抑制了土壤脲酶活性的提高,而脲酶活性在培养结束时仍维持在高活性水平则可能受到底物降解速率的影响[28].亚硝酸还原酶活性在培养初期均有显著升高,并随着时间进程逐步降低.这是由于初期的培养过程硝酸还原酶促进了反硝化作用,为亚硝酸还原酶提供了充足的底物(图 3).当然,这些过程是同时发生,且相互反馈和相互刺激的,在短期的培养试验并未得到完整的体现.这进一步说明萘作为驱虫剂的非目标效应,

可能在短期内对川西亚高山森林土壤的氮循环过程产生重要的影响.

短期培养试验显示,萘对川西亚高山森林土壤生化特性产生了不同程度的影响.萘作为驱虫剂的非目标效应,能在短期内增加土壤呼吸速率,改变土壤可溶性氮和有效氮含量的动态,抑制土壤脲酶活性,但对土壤可溶性碳含量,以及蔗糖酶、硝酸还原酶和亚硝酸还原酶活性影响不明显.与土壤碳循环相比,萘可能在短期内对川西亚高山森林土壤的氮循环过程产生更加强烈的影响.值得注意的是,由于室内模拟的局限性,缺乏地上部分植被的吸收/周转参与.这种存在非目标效应在野外控制试验中是否同样存在,值得深入研究.

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