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生物炭对农田土壤中氮循环及氧化亚氮产生的影响与机理

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摘 要

氧化亚氮(N_2O)作为温室气体和臭氧层破坏者备受学者关注，其中农田土壤是非人为条件下N_2O最主要的排放源。土壤中参与氮素循环的生物/非生物过程中复杂多变，探索不同路径的氮转化机制以及对N_2O的贡献度有助于对N_2O减排提供机理剖析。生物炭因其高孔隙度、强吸附性、化学稳定性和大阳离子交换量等优点，会对土壤中氮素的转化产生直接/间接的影响，并显著改善/恶化土壤N_2O排放。因此，总结了生物炭对土壤生态系统中氮素的转化与N_2O排放的研究现状，分别论述了生物炭对无机氮循环与N_2O排放的影响，并从生物炭吸附、影响土壤理化性质、群落结构多样性以及关键功能基因等方面揭示了其作用机制。基于以上内容，对今后生物炭在N_2O增汇减排领域的进一步理论研究和相关技术推广进行了展望。

关键词

N_2O，氮循环，硝态氮，铵态氮，生物炭

Effect and Mechanism of Biochar on Nitrogen Cycle and Nitrous Oxide Production in Farmland Soil

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Abstract

Nitrous oxide (N_2O) as a greenhouse gas and ozone layer destroyer has attracted much attention from scholars, and farmland soil is the main source of N_2O emissions under non-human conditions. The biotic/abiotic processes involved in the nitrogen cycle in soil are complex and changeable. Exploring the nitrogen transformation mechanism of different pathways and the contribution to N_2O will help provide a mechanism analysis for N_2O emission reduction. Due to its advantages of high porosity, strong adsorption, chemical stability and large cation exchange capacity, biochar can have direct/indirect effects on nitrogen transformation in soil and significantly improve/worse soil N_2O emissions. Therefore, this paper summarizes the research status of biochar on nitrogen transformation and N_2O emission in soil ecosystems, discusses the effects of biochar on inorganic nitrogen cycle and N_2O emission, and discusses the effects of biochar adsorption, soil physical and chemical properties, and community structure. Diversity as well as key functional genes revealed its mechanism of action. Based on the above content, the further theoretical research and related technology promotion of biochar in the field of N_2O sink emission reduction in the future are prospected.

Keywords

N_2O, Nitrogen Cycle, Nitrate Nitrogen, Ammonium Nitrogen, Biochar

1. 引言

作为六类温室气体之一的氧化亚氮(N_2O)，其温室效应是 CO_2 的 298 倍，同时还参与了臭氧层的破坏，对生态环境和人类健康都有着巨大威胁[1]。自工业革命以来，全球大气 N_2O 平均浓度从~270 ppb 增长到了 2021 年的 618 ppb。其中，土壤是大气中 N_2O 的主要来源，从工业革命前的 6.3 ± 1.1 Tg N·a^{-1} 上升到了 2020 年的 17.0 ± 2.0 Tg N·a^{-1} [2]。学者们普遍认为，土壤中微生物对氮的转化过程是 N_2O 的最主要来源。然而，随着对 N_2O 排放机制的逐步深入探究，发现硝化细菌反硝化、异养反硝化以及硝化耦合反硝化等生物作用以及羟胺氧化和化学反硝化等非生物作用也可产生 N_2O，且多个过程往往同时发生[3]。尽管已经开展了不少研究，但目前对不同环境条件或不同类型土壤 N_2O 产生路径贡献及其主控因素的认识仍非常不足。

生物炭(biochar)，即生物质在缺氧条件下经高温热裂解产生的富碳产物，能有效吸附铵态氮(NH_4^+-N)与硝态氮(NO_3^--N)，并显著影响土壤的理化性质[4]，进而影响到参与氮循环有关微生物群的多样性与丰度，最终对土壤氮循环产生影响[4]。生物炭的微孔结构可以为微生物繁殖提供载体，使它们免遭干燥等不利条件的影响。同时还可以吸附大量水分、养分物质，为微生物提供营养物质。另外，生物炭可以调控土壤的理化性质，进而影响微生物的新陈代谢，这使得生物炭对土壤微生物的影响呈现出复杂性，其作用机制尚未研究清楚。

因此，首先综述了农田土壤中 N_2O 的可能产生途径，便于深入了解 N_2O 排放的主要来源，为有效的 N_2O 减排举措提供理论依据。其次，介绍了近年来农田土壤中生物炭对微生物的无机氮转化过程以及 N_2O 排放影响的研究进展。最后，概括了生物炭对 N_2O 排放机制的影响机制。基于以上研究基础，提出未来

展望：i) 生物炭特征数据库的丰富；ii) 生物炭在 N_2O 减排潜力估算的精确性；iii) 长期原位田间试验，为农田土壤生态系统的 N_2O 减排提供理论参考。

2. 农田土壤中 N_2O 的产生途径

2.1. 生物过程中 N_2O 的产生

土壤中 N_2O 的排放主要来源于微生物有关的氮转化过程，即自养/异养硝化、硝化细菌反硝化、硝化耦合反硝化以及异养反硝化等过程。

2.1.1. 自养硝化过程

自养硝化也称氨氧化，即自养硝化细菌在有氧条件下利用 CO_2 作碳源，使 NH_3 氧化为 NO_3^--N 的过程，而 N_2O 作为副产物被排放[5]。自养硝化过程主要分两步完成，氨氧化细菌(AOB)/氨氧化古菌(AOA)利用氨单加氧酶(AMO)将 NH_3 催化生成 NO_2^--N。随后亚硝酸氧化细菌(NOB)利用亚硝酸氧化酶(NXR)将 NO_2^--N 氧化成 NO_3^--N。AOB 产生 N_2O 主要有两种方式，一种方式是羟胺氧化酶(HAO)将羟胺(NH_2OH)和一氧化氮还原酶(NOR)的作用下为 N_2O [6]。第二种方式是 AOB 通过亚硝酸还原酶(NIR)和 NOR 将 NO_2^--N 还原为 N_2O [7]。也有研究表明，AOB 可以通过细胞色素 P460 直接将 NH_2OH 或 NO 氧化为 N_2O [8]。与 AOB 不同，AOA 因缺乏 HAO 和 NOR，导致其产生 N_2O 主要通过 NH_2OH 与 NO 的非生物耦合过程。但最新结果表明，AOA 可以通过细胞色素 P450 将 NO_2^--N 还原为 N_2O，说明 AOA 也可以进行反硝化作用[9]。研究发现，环境中存在一种能将 NH_3 直接氧化成 NO_3^--N 的微生物，被称之为“完全氨氧化细菌”(comammox*Nitrospira*)，但由于缺少 NOR，comammox*Nitrospira* 产生 N_2O 的主要方式为 NH_2OH 的非生物转化[10]。

影响自养硝化过程对 N_2O 产生贡献的因素有 pH 值、碳氮比(C/N)、温度和 O_2 等[11]。研究表明，自养硝化过程占碱性土壤 N_2O 排放的 65%~86%，但对于酸性土壤，则仅占 5%~25%。这主要是因为酸性土壤中 AOA 在硝化过程起到主要作用，而在中性/碱性土壤中 AOB 对 N_2O 产生的贡献会更大[12]。土壤中 O_2 下降能够抑制自养硝化对 N_2O 产生的贡献[13]。目前关于温度如何影响自养硝化过程中 N_2O 产生尚未达成一致。由于自养硝化过程是整个氮循环的初始步骤，明确其影响因素有助于我们理解 N_2O 排放对气候变化的反馈机制。

2.1.2. 异养硝化过程

异养硝化过程是指微生物将有机氮氧化为 NO_3^--N/ NO_2^--N 的过程，其与反硝化联系紧密，在土壤中普遍存在[14]。在特定条件下异养硝化过程能产生大量的 N_2O。低 pH 时自养硝化微生物活性常常被抑制，导致异养硝化为 N_2O 产生的主要过程[15]。但也有研究表明，异养硝化过程对 N_2O 的产生贡献跟土壤 pH 值无关，而与土壤 C/N 比、含水量以及全氮含量的关系更密切[16]。Tang 等[17]的研究表明，土壤含水量上升会导致异养硝化过程产生的 N_2O 通量显著增加。另一项研究表明，厌氧环境会降低异养硝化过程中 N_2O 的产生。温度对异养硝化过程 N_2O 的产生影响研究还相对较少[18]。Jansen-Willems 等[19]的研究证明，适当的提高温度能够促进异养硝化过程中 N_2O 的产生。由于气温升高可能会通过促进土壤有机质的分解为异养微生物提供碳源，因此，升温很可能也会促进其他生态系统异养硝化过程产生 N_2O，但这仍需要进一步的研究。

2.1.3. 硝化细菌反硝化过程

硝化细菌反硝化是 NO_2^--N 通过 NO 还原为 N_2O 的过程。农田土壤中低 O_2 条件下，硝化细菌反硝化对 N_2O 排放的贡献达 72.7% [16]。也有研究表明，硝化细菌反硝化过程广泛存在于农田土壤中，并且是

N_2O 产生的主要路径[20]。硝化细菌反硝化在土壤中的发生主要受到土壤含水量、pH 值、O_2 浓度、土壤有机碳(SOC)和 NO_2^--N 含量等条件的影响[21]。Wrage-Mönnig 等[22]证明了硝化细菌反硝化过程在低 O_2、低碳浓度以及低 pH 值条件下更容易发生。但也有研究表明，对于高 pH 值土壤，硝化细菌反硝化在 N_2O 产生中的作用更大[23]。Duan 等[24]在农田土壤的研究表明，温度对硝化细菌反硝化产生 N_2O 的影响因土壤类型而异，如温度对酸性土壤中硝化细菌反硝化过程 N_2O 的产生没有显著影响，但增加了碱性土壤中硝化细菌反硝化过程 N_2O 的产生。以后的研究需要更多关注硝化细菌反硝化对土壤 N_2O 产生的贡献及其控制因子。此外，关于硝化细菌反硝化是否为 N_2O 的汇(还原为 N_2)以及 AOA 是否能参与此过程，也需要加强研究。

2.1.4. 硝化耦合反硝化过程

硝化耦合反硝化通常是指在硝化过程所产生的 NO_3^--N/ NO_2^--N 被反硝化细菌迅速利用而最终产生 N_2 的过程[25] [26]。硝化耦合反硝化过程一般在有氧和缺氧共存的微环境条件下对 N_2O 产生的贡献较高，如土壤中颗粒表面的水膜界面、龟裂土壤边缘或者土壤干湿交替条件[27]。Verhoeven 等[28]发现，干湿交替的土壤中硝化耦合反硝化的 N_2O 贡献幅度高达 34.8%。土壤 pH 和底物浓度等是影响硝化耦合反硝化对 N_2O 产生的主要因素。有研究表明，高 pH 值对硝化耦合反硝化产生 N_2O 的贡献更高。但 Kool 等[29]研究表明，硝化耦合反硝化对 N_2O 产生的贡献随土壤 pH 值增加而降低。此外，Duan 等[23]研究表明，由于土壤可溶性有机碳(DOC)能促进 N_2O 还原为 N_2，硝化耦合反硝化对 N_2O 产生的贡献随土壤 DOC 浓度的增加而降低。

2.1.5. 异养反硝化过程

异养反硝化是细菌将 NO_3^--N 逐步还原为 NO_2^--N、NO、N_2O 和 N_2 的过程[30] [31]。研究表明，在全球尺度上，来源于异养反硝化的 N_2O 占反硝化过程 N_2O 产生总通量比例介于 6%~11% [32]。含水量的变化对异养反硝化中 N_2O 产生贡献较大，在高含水量条件下对异养反硝化 N_2O 产生贡献较大[33]。氮源、C/N 比、O_2 以及 pH 值等也会影响异养反硝化过程 N_2O 的产生速率[34]。Qu 等[35]的结果表明，施肥的过度施用导致了土壤酸化，反硝化产物 $N_2O/(N_2O+N_2)$ 比值也随着增加。

土壤 SOC 含量通常决定着真菌反硝化对 N_2O 的贡献。Li 等人研究表明，耕地转变为茶园之后，由于 SOC 含量增加，真菌对 N_2O 产生贡献相应增加[36]。土壤高 NO_3^--N 浓度、较低的 O_2 以及较高的温蒂能显著刺激真菌反硝化过程[37]。但也有研究表明，低温也能促进真菌反硝化对 N_2O 产生的贡献[38]。真菌对 O_2、pH 值、温度和底物(NO_3^--N/ NO_3^--N)等的适应范围很广[39]。因此，真菌反硝化对土壤 N_2O 产生贡献不可忽视，需要深入关注。

2.2. 非生物过程中 N_2O 的产生

越来越多的证据显示，在一定条件下，非生物过程可能是土壤 N_2O 产生的重要路径[40]。 NO_3^--N、NO_2^--N 以及 NH_2OH 驱动非生物过程中 N_2O 的产生主要是通过光分解和铁离子还原作用， NO_2^--N 和还原性金属离子(如 Fe^{3+}和 Mn^{2+})反应、NH_2OH 和有机质以及 Fe^{3+}/MnO_2 的反应[41]。

NO_2^--N 通过非生物降解生成 N_2O 的过程被称为化学反硝化[41]。尽管参与此过程的 NO_2^--N 是由生物过程产生，但是以往针对化学反硝化的研究多关注其非生物反应环节，而未将生物和非生物过程当作整体考虑。随着研究的深入，生物和非生物的耦合过程逐渐受到重视。如 Onley 等[42]通过纯培养实验发现，尽管 *Anaeromyxobacterdehalogenans* 细菌不含 *nirK* 和 *nirS* 基因，但在 Fe^{2+}存在条件下可以通过生物和非生物耦联过程产生 N_2O。另一项纯培养研究也表明，由 AOA、AOB 和 comammox*Nitrospira* 所产生的 NH_2OH 释放到细胞外后能通过非生物过程产生 N_2O [43]。与 NO_2^--N 相比，NH_2OH 参与的非生物过程

中 N_2O 的生成效率更高。在低 pH 或 Fe^{3+}、有机质(SOM)以及 NO_2^--N 浓度较高的条件下，NH_2OH 经由非生物过程生成 N_2O 的效率可达 40%~80% [44]。

土壤 N_2O 的非生物产生过程与 pH 值、SOM、Fe/Mn 含量等因素有关[45]。有研究表明，在低 SOC 和高铁离子含量的土壤条件下，能够促进 NH_2OH 向 N_2O 的非生物转化[46]。但最新的结果表明，NH_2OH 向 N_2O 的非生物转化过程与土壤 Fe^{3+}、Mn^{2+}、SOC 和总氮含量无关，而与土壤 pH 值呈正相关关系[47]。NH_2OH 一般在土壤中难以被检测到。这主要是因为 NH_2OH 既通过生物和非生物过程快速生成 N_2O，又能通过非生物过程被 SOM 固定，还可以通过硝化过程被快速氧化成 NO_3^--N。因此，今后需加强对生物－非生物耦合过程 N_2O 产生的研究，以期深入揭示土壤 N_2O 产生与消耗过程。

3. 生物炭对农田土壤中氮循环的影响

生物炭对无机氮(即 NH_4^+-N 和 NO_3^--N)的吸附程度是有差异的。Yin 等人[48]的研究表明，生物炭与畜禽堆肥一起施用后，能让土壤中 NH_3 的挥发损失降低至少一半。Sun 等[49]发现，生物炭(20 t·hm^{-2})与氮肥(250 kg·hm^{-2})的共同施用抑制了水稻土壤中 36.6%的 NH_3 挥发量，促进了 30.1%的氮肥利用率，提升了 55.6%的小麦产量。Chu 等[50]发现，在酸性茶园土壤中的生物炭可以显著减少 28.21%的 NH_3 挥发量。进一步的研究表明，生物炭比表面积大、富含酸性官能团以及表面负电荷多，对土壤中的 NH_4^+-N 和 NO_3^--N 具有较强吸附能力[51]。

土壤淋溶是造成养分流失的罪魁祸首[52]，而众多实验表明生物炭能够显著缓解淋滤造成的氮损失[53]。Cao 等[54]发现：30 t·hm^{-2} 的生物炭明显降低了砂质土壤中 NH_4^+-N (14%)和 NO_3^--N (28%)的淋溶损失。Borchard 等[55]发现，生物炭所降低的 NH_4^+-N 和 NO_3^--N 与炭土质量比呈现正相关关系，当炭土质量比分别为 0.5%、2.5%和 10.0%时， NH_4^+-N 和 NO_3^--N 的淋溶损失分别降低 14%、50%和 89%和 26%、42%和 96%。生物炭类型的差异也会导致对氮素固留效果的不同。与稻草生物炭相比，秸秆生物炭和毛竹生物炭分别明显降低了 74.8%和 31.6%的总氮淋失[56]。由以上结果可知，生物炭通过改善土壤理化性质(增大颗粒间孔隙度、降低容重等)，可以增强对氮素的固持作用，从而达到缓解土壤氮素淋失的目的[57]。

3.1. 生物炭对农田土壤中硝化作用的影响

土壤中的微生物将 NH_4^+-N 转化为 NO_2^--N/ NO_3^--N 的过程称为硝化作用，依据碳源种类可分为自养硝化和异养硝化[58]。通常认为，自养硝化是土壤中 N_2O 排放的主要贡献者[59]。自养硝化过程中会产生不稳定的 NH_2OH 作为中间产物，之后通过化学分解/酶促反应生成 N_2O [60]。自养硝化由氨氧化和亚硝化两部分组成。其中，氨氧化作用在 AOA/AOB 中的 AMO 和 HAO 的依次催化下实现 NH_3 的氧化过程，N_2O 作为副产物被排放[61]。生物炭添加会明显影响 AOA/AOB 的丰度和多样性。而 AOA/AOB 在土壤、湿地和海洋等生态系统的氮循环中均发挥着举足轻重的作用[62]。当土壤中的氧分压、温度、pH 值、含水量、养分等任一因素变化时，AOA/AOB 的丰度和活性都会产生较大波动[63]。Zhang 等[64]发现富含生物炭的土壤中 AOA 的基因拷贝数比对照高出约 50%。Wang 等[65]的研究表明 5%、10%和 20%的生物炭在沿海碱性土壤中被施用后，AOB 的丰度分别增加了 15.9%、121.0%和 28.6%，但 AOA 无显著变化。然而，也有研究发现生物炭对土壤氨氧化作用具有一定的抑制效果[66]。Ahmed 等[67]将 20 t·hm^{-2} 的生物炭和 760 kg·hm^{-2} 的氮肥一起施用后，结果发现土壤硝化率明显受到了抑制，机理研究表明生物炭能够释放一种“α 松萜”的硝化抑制剂到土壤中，从而影响了 N_2O 和 NH_3 的产生。DELUCA 等[53]研究表明，生物炭通过吸附固定 NH_4^+-N 从而抑制了森林土壤中的氨氧化作用[68]。由以上可知，土壤中的生物炭对氨氧化作用的影响机制主要有以下三点：i) 生物炭改变土壤的 pH 值、氧分压、团聚体和孔隙度等特性，进而影响氨氧化功能微生物的多样性和丰度[69]；ii) 生物炭固持土壤中的 NH_4^+-N，导致氨氧化作用的减弱[70]；iii) 生物炭产生硝化抑制剂，减弱了氨氧化作用[71]。

3.2. 生物炭对农田土壤中反硝化作用的影响

在土壤反硝化过程中建立排放比(N_2O/N_2)这个参数，其往往受到众多环境因子的影响[72]。*NosZ*、*nirK*、*nirS* 是反硝化细菌中研究中最多的 3 类功能基因，它们的丰度和多样性的改变可以作为生物炭对反硝化影响的指示因子。Aamer 等[73]的研究表明，生物炭显著增强了 nosZ/nirK，降低了 N_2O/N_2。Liu 等[74]结果证实质量分数分别为 1%、2%和 10%的生物炭均能提升反硝化功能基因的丰度。基于已报道的文章，生物炭对土壤反硝化作用的影响机制主要包括，生物炭具有多孔的特性可以改善土壤通气状况，提升好氧转化酶的活性[75]。生物炭自身丰富的官能团改变了土壤 pH 值，影响反硝化的不同酶活性[76]。生物炭中的有机物质对反硝化微生物的群落结构及功能基因的多样性与丰度造成影响[77]。

3.3. 生物炭对农田土壤中 N_2O 排放的影响

由于制备原料、土壤质地、施用时间、气候类型和田间管理方法等条件的不同，生物炭对 N_2O 排放的影响也不同[62]。一般来说，木质炭可以减少 N_2O 的排放，但畜禽粪便炭却没有这种效果[63]。在强酸土壤中生物炭对减少 N_2O 排放的作用比中性土壤要小很多[64]。Wang 等人[65]发现，应用生物炭可显著减少土壤 28.8%~31.3%的 N_2O 排放，其关键机制是生物炭降低了土壤活性氮的浓度和氮循环相关酶的活性。Zhang 等[71]还发现，添加 1%的生物炭可以显著提高 *nirS* 和 *nosZ* 基因的丰度，增加 $N_2/(N_2O + N_2)$，促进 N_2O 完全还原为 N_2。基于以上结构，生物炭抑制土壤 N_2O 排放的机制被提出，即生物炭明显改善土壤通气性，抑制土壤反硝化过程[65]；生物炭通过吸附 NH_4^+-N，降低硝化作用从而抑制 N_2O 的排放[75] [76]；生物炭通过改善 pH，提升 N_2O 还原酶的活性[77]。

4. 总结与展望

总而言之，氮素转化与 N_2O 排放是土壤生态系统中氮循环的主要组成部分。农田土壤有多条 N_2O 产生路径，包括自养硝化、异养硝化、硝化细菌反硝化、硝化耦合反硝化、异养反硝化以及化学反硝化等生物/非生物过程。这些路径之间相互关联，受多种环境因素的共同制约。但现有的研究成果主要基于实验室规模，不能完全还原原位土壤中实际发生的过程。同样，生物炭对土壤中 N_2O 排放的作用与机制也十分复杂，均会受到生物炭类型、土壤类型、炭土施用比以及 N_2O 测定精度等环境因素的限制，基于此，对不同类型生物炭应用于不同类型土壤的长期原位户外实验的研究迫在眉睫。

当前关于生物炭对土壤氮素转化的影响机制分析大多聚焦于生物炭自身或导致土壤理化性质的改变，而对土壤氮循环中的功能微生物多样性和丰度的研究鲜被报道。一部分学者认为生物炭通过吸附碳源导致了土壤中能产生 N_2O 的微生物碳源利用减少，另一部分学者则认为生物炭通过改变功能微生物的种类来实现抑制 N_2O 的排放。因此，对氮素转化和 N_2O 排放的微生物生态机制的研究可能是未来土壤生态学的重点。

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