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禾本科作物联合固氮研究进展

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摘要: 生物固氮是唯一能将空气中“免费”的氮气转化为化合态氮的生物学过程。一般认为豆科作物具有共生固氮能力，间套种豆科作物已成为补充农田氮素的重要方式。越来越多的证据证明禾本科作物也具有较高的联合固氮潜力，大量联合固氮菌不仅定殖在根际、根内，还可以定殖在植株地上部如茎维管束、叶际中，表明禾本科作物固氮微生物可能为避免复杂的土壤环境，开辟了一条“体内高效固氮”的新途径。本文回顾了近年来玉米、小麦、水稻、甘蔗等禾本科作物在联合固氮部位、调控途径、菌群构建等方向取得的创新进展，重点介绍了固氮菌除了与宿主植物存在互作关系外，还与其他功能细菌、真菌和病毒之间存在潜在的相互作用。基于生物固氮多功能合成菌群在植物营养和促生等领域表现出的巨大应用前景和潜力，提出了当前禾本科作物联合固氮研究的前沿热点和难点，即如何综合利用“自上而下”和“自下而上”策略，筛选关键功能类群并结合基因组尺度代谢模型，构建群落稳定、功能多样、效果显著的合成菌剂，为生物固氮在农业生产中广泛应用提供强有力的技术支撑。

关键词: 禾本科作物；生物固氮；固氮菌；秸秆分解；合成菌群

Research progress on associative nitrogen fixation of gramineous crops

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Abstract: Biological nitrogen fixation (BNF) is the only biological process that can convert the“free”atmospheric nitrogen into chemical nitrogen for the utilization of plant and microorganism. Leguminous crops are widely known about their high efficiency in symbiotic nitrogen fixation, and have been used in agriculture as a supplement of nitrogen for other crops. Recent studies found that gramineous crops also had powerful associative nitrogen fixation capacity. In particular, large numbers of associative nitrogen-fixing bacteria have been identified in root, rhizosphere, and the above ground compartments of plants, like stem vascular bundle and phyllosphere, suggesting that nitrogen-fixing microorganisms in gramineous crops may have created a new pathway for“efficient nitrogen fixation *in vivo*”to avoid complex soil environments. This article presents an overview of the recent advancements in the associative nitrogen fixation compartments, regulatory pathways, and microbial community construction of gramineous crops, such as maize, wheat, rice, and sugarcane. This review focuses on the potential interactions between nitrogen-fixing bacteria and other functional bacteria, fungi, and viruses, beyond their interactions with host plants. Based on the promising application prospects of multifunctional synthetic microbial community in the fields of plant nutrition and growth promotion, this paper outlines the current research frontiers and challenges in

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associative nitrogen fixation of gramineous crops. The ultimate aim is to integrate the“top-down”and“bottom-up”strategies to identify key functional groups, combine genome-scale metabolic models, and construct stable, functionally diverse, and effective synthetic microbial community, and to provide robust technical support for the widespread application of BNF in agricultural production.

Key words: gramineous crops; biological nitrogen fixation; nitrogen-fixing bacteria; straw decomposition; synthetic community

氮是植物生长所需的大量营养元素之一。空气中游离态的氮气约占空气成分的 79%，然而绝大多数作物不能直接利用大气中“免费”的氮气。生物固氮 (biological nitrogen fixation, BNF) 是指氮气在固氮菌的参与下被转化为氨的过程。根据固氮微生物与宿主植物的共生关系，可将生物固氮分为共生固氮、联合固氮和自生固氮[1–3]，其中联合固氮与植物形成一种松散的互利共生关系，但不形成类似豆科植物共生固氮的根瘤结构。生物固氮在自然界中广泛存在，既是作物产量与氮素供应的增强剂，又是化学氮肥减量和温室气体减排的有效途径，其重要程度不亚于光合作用。

据估算，全球生物固氮量每年超过两亿 t，其中海洋约占 2/3，陆地约占 1/3[4–5]。当前，农田生态系统主要依靠化学氮肥的投入，以满足粮食作物种植过程中的氮素需求。实际上，生物固氮同样是农作物–土壤系统中氮养分输入的关键源头之一，总量达到 32 Tg，占氮素总输入量的 18.93%[6]。尽管豆科作物根瘤菌等共生固氮具有固氮效率高的特性，占 59.17% 的农田生物固氮总量[7]，但大量研究表明禾本科作物同样具有联合固氮能力[3, 8–10]，其单位固氮效率虽然低于豆科作物，但是禾本科作物种植面积远高于豆科作物，所以固氮潜力同样巨大。通过不同文献估算全球玉米、水稻、小麦平均固氮效率约为 N 26.51 kg/hm^2，根据种植面积可得全球固氮量达到 15.58 Tg，而大豆、花生等豆科作物固氮效率大约为每年 N 109.00 kg/hm^2，其全球固氮量为 17.91 Tg[7, 11]。因此，从全球角度来看禾本科作物生物固氮量与豆科作物固氮量相当，但禾本科作物生物固氮的供应潜力被低估。

为了满足全球人口日益增长带来的粮食需求量增加，未来小麦、玉米、水稻等主要粮食作物的氮需求量可能会进一步增加。化学氮肥的投入增加会进一步引起土壤盐化酸化、温室气体增排等系列负面效应，禾本科作物生物固氮将成为未来最为绿色的替代氮源。因此，本文以禾本科作物为对象，从禾本科作物固氮微生物类群与功能及其关键部位、禾本科作物生物固氮调控途径、禾本科作物秸秆分解与生物固氮互作关系、生物固氮多功能合成菌群构建与应用等角度进行了系统阐述，旨在提升禾本科作物生物固氮潜力，优化禾本科作物–土壤系统氮素循环过程。

1 禾本科作物联合固氮微生物类群与功能

1961 年，Dobereiner[12]在甘蔗根际分离获得拜叶林克氏菌属 (*Beijerinckia*) 的固氮菌，证实了禾本科作物具备生物固氮潜力，之后在禾本科作物联合固氮菌研究中陆续发现了假单胞菌属 (*Psedomonas*)、克雷伯氏菌属 (*Klebsiella*)、肠杆菌属 (*Enterobacter*) 等菌株。禾本科作物联合固氮菌既可直接固氮供作物吸收，也可通过多种代谢途径调节、刺激外源氮素的转化、代谢，提高作物对氮素的吸收利用能力。近年来禾本科作物联合固氮菌的类群与功能见表 1。

1.1 玉米联合固氮

玉米与固氮菌之间存在显著的互作关系。研究人员将一株假单胞固氮菌 (*Pseudomonas stutzeri* A1501) 接种玉米后发现，植株生物量和氮含量分别增加 25.4% 和 7.8%，且 A1501 同时具备生物固氮和产生植物激素功能，接种固氮菌 A1501 显著促进了玉米生长[13]。从玉米根系分离获得的固氮菌 (*Kosakonia sacchari* PBC6.1) 可在低氮条件下通过谷氨酰胺传感蛋白 (GlnD) 修饰 PII 蛋白，从而达到固氮途径上调目的[14]。Montañez 等[15]从玉米根茎叶中分离出大量泛菌属 (*Pantoea*)、假单胞菌属 (*Pseudomonas*)、草螺菌属 (*Herbaspirillum*) 等固氮菌，它们为玉米提供了 12%～33% 的氮源。最近研究表明，玉米茎木质部存在一个以 γ-变形菌门 (Gamma-proteobacteria) 为主的固氮微生物组，并且茎木质部微生物组携带的 *nifH* 基因丰度明显高于根、叶等其他部位，这些固氮菌为玉米提供了重要的氮源[3]。

1.2 小麦联合固氮

接种固氮菌是提高小麦产量的有效途径之一。固

表 1　已知玉米、小麦、水稻和甘蔗相关联合固氮菌的类群与功能

Table 1　Reported associative nitrogen-fixing bacteria species and functions of maize, wheat, rice and sugarcane

作物 Crop	门 Phylum	属 Genus	功能 Function	参考文献 Reference
玉米 Maize	放线菌门 Actinobacteria	微杆菌属、微球菌属 *Microbacterium*, *Micrococcus*	生物固氮、产生植物激素、产生铁载体 Biological nitrogen fixation, production of plant hormones and iron carriers	[33−34]
	α-变形菌门 Alphaproteobacteria	固氮螺菌属、柄杆菌属 *Azospirillum*, *Caulobacter*	生物固氮 Biological nitrogen fixation	[35−36]
	拟杆菌门 Bacteroidota	金黄杆菌 *Chryseobacterium*	生物固氮 Biological nitrogen fixation	[33]
	β-变形菌门 Betaproteobacteria	伯克霍尔德菌、草螺菌属、螺菌属 *Burkholderia*, *Herbaspirillum*, *Spirillum*	生物固氮、产生植物激素、溶解磷酸盐、抵抗病原菌 Biological nitrogen fixation, production of plant hormones, dissolvement of phosphates, resistance to pathogens	[15, 37−40]
	厚壁菌门 Firmicutes	芽孢杆菌、赖氨酸芽孢杆菌属、类芽孢杆菌属、葡萄球菌属 *Bacillus*, *Lysinibacillus*, *Paenibacillus*, *Staphylococcus*	生物固氮、产生植物激素、溶解磷酸盐、抵抗病原菌 Biological nitrogen fixation, production of plant hormones, dissolvement of phosphates, resistance to pathogens	[33, 40−44]
	γ-变形菌门 Gammaproteobacteria	柠檬酸杆菌属、肠杆菌属、克雷伯氏菌属、科萨克氏菌属、泛菌属、假单胞菌属 *Citrobacter*, *Enterobacter*, *Klebsiella*, *Kosakonia*, *Pantoea*, *Pseudomonas*	生物固氮、产生植物激素、产生铁载体、溶解磷酸盐、抵抗病原菌、增加植物生物量、调控植物基因表达 Biological nitrogen fixation, production of plant hormones and iron carriers, dissolvement of phosphates, resistance to pathogens, plant biomass increment, regulation of plant gene expression	[13−15, 37, 40, 45−46]
小麦 Wheat	α-变形菌门 Alphaproteobacteria	固氮螺菌属 *Azospirillum*	生物固氮、调控植物基因表达、增强养分吸收效率 Biological nitrogen fixation, regulation of plant gene expression, enhancement of nutrient uptake efficiency	[16, 21, 36]
	厚壁菌门 Firmicutes	类芽孢杆菌属 *Paenibacillus*	生物固氮、增加植物生物量、调控植物基因表达、增强养分吸收效率 Biological nitrogen fixation, plant biomass increment, regulation of plant gene expression, enhancement of nutrient uptake efficiency	[17, 20]
	γ-变形菌门 Gammaproteobacteria	肠杆菌属、假单胞菌属 *Enterobacter*, *Pseudomonas*	生物固氮、产生植物激素、产生铁载体 Biological nitrogen fixation, production of plant hormones and iron carriers	[16, 18, 47]
水稻 Rice	α-变形菌门 Alphaproteobacteria	固氮螺菌属、慢生根瘤菌属 *Azospirillum*, *Bradyrhizobium*	生物固氮、调控植物基因表达、增加植物生物量 Biological nitrogen fixation, regulation of plant gene expression, enhancement of plant biomass	[23−24, 48−49]
	β-变形菌门 Betaproteobacteria	草螺菌属、帕拉伯克霍尔德氏菌属、丙酸弧菌属 *Herbaspirillum*, *Paraburkholderia*, *Propionivibrio*	生物固氮 Biological nitrogen fixation	[37, 48, 50]
	蓝藻门 Cyanobacteriota	念珠藻属 *Nostoc*	生物固氮 Biological nitrogen fixation	[51]
	δ-变形菌门 Deltaproteobacteria	*Fundidesulfovibrio*	生物固氮 Biological nitrogen fixation	[52]
	厚壁菌门 Firmicutes	芽孢杆菌属、类芽孢杆菌属 *Bacillus*, *Paenibacillus*	生物固氮、产生植物激素 Biological nitrogen fixation, production of plant hormones	[53−54]
	γ-变形菌门 Gammaproteobacteria	肠杆菌属、克雷伯氏菌属、食二氮植物杆菌、假单胞菌属 *Enterobacter*, *Klebsiella*, *Phytobacter*, *Pseudomonas*	生物固氮、产生植物激素、增强植物养分利用效率、调控植物基因表达 Biological nitrogen fixation, production of plant hormones, enhancement of nutrient uptake efficiency, regulation of plant gene expression	[24, 37, 53, 55]

续表 1 Table 1 continued

作物 Crop	门 Phylum	属 Genus	功能 Function	参考文献 Reference
甘蔗 Sugarcane	α-变形菌门 Alphaproteobacteria	醋杆菌属、拜叶林克氏菌属、葡糖醋杆菌属、固氮螺菌属 *Acetobacter*, *Beijerinckia*, *Gluconacetobacter*, *Nitrospirillum*	生物固氮、产生植物激素、产生铁载体 Biological nitrogen fixation, production of plant hormones and iron carriers	[12, 37, 56−57]
	厚壁菌门 Firmicutes	芽孢杆菌属 *Bacillus*	生物固氮、抵抗病原菌 Biological nitrogen fixation, resistance to pathogens	[58]
	β-变形菌门 Betaproteobacteria	伯克霍尔德菌 *Burkholderia*	生物固氮、抵抗非生物胁迫 Biological nitrogen fixation, resistance to abiotic stresses	[59]
	γ-变形菌门 Gammaproteobacteria	肠杆菌属、克雷伯氏菌属、科萨克氏菌属、泛菌属、假单胞菌属、寡养单胞菌属 *Enterobacter*, *Kelbsiella*, *Kosakonia*, *Pantoea*, *Pseudomonas*, *Stenotrophomonas*	生物固氮、产生植物激素、产生铁载体、抵抗非生物胁迫、溶解磷酸盐、调控植物基因表达 Biological nitrogen fixation, production of plant hormones and iron carriers, resistance to abiotic stresses, dissolvement of phosphates, regulation of plant gene expression	[29, 31−32, 60−63]

氮螺菌属 (*Azospirillum*)、类芽孢杆菌属 (*Paenibacillus*)、假单胞菌属 (*Pseudomonas*)、肠杆菌属 (*Enterobacter*) 是与小麦密切相关的联合固氮类群，对小麦氮获取有很大贡献[16−19]。例如，小麦内生类芽孢杆菌在低氮肥条件下能显著增加 86.1% 植株地上部干重，氮摄取和代谢基因在接种类芽孢杆菌后表达量上调 1.5～91.9 倍，进而提高了小麦养分吸收能力[20]。巴西固氮螺菌可以有效地在小麦根系定殖，并上调小麦根系硝酸盐转运蛋白 (NAXT, PTR) 基因表达，小麦根系转录谱的改变促进了幼苗根系伸长和养分获取能力[21]。

1.3 水稻联合固氮

固氮微生物对水稻生长十分重要[22]。巴西固氮螺菌 (*Azospirillum brasilense*) 是水稻根系促生菌，该菌定殖水稻根系过程中可引起根系转录因子、蛋白激酶和转运蛋白相关基因的差异表达，从而通过调控根系类黄酮合成、激素信号转导等过程促进水稻生长[23]。在水稻上的田间试验结果表明，3 株慢生根瘤菌属 (*Bradyrhizobium*) 固氮菌均不同程度地增加了水稻干重、产量和千粒重，尤其是埃氏慢生根瘤菌 (*Bradyrhizobium elkanii* SEMIA 587) 使得水稻增产 1000 kg/hm^2，扩增子分析表明这些埃氏慢生根瘤菌主要集中在水稻地上部，在提升叶片叶绿素含量和增强光合作用方面发挥了重要作用[24]。一组包含假单胞菌属 (*Pseudomonas*) 和红酵母属 (*Rhodotorula*) 固氮菌群定殖水稻后，明显改善水稻氮含量和氮利用效率，固氮菌定殖 24 h 内水稻根系氮代谢、氮转运和诱导根系结节启动表达基因上调[25]。研究表明，固氮菌一方面通过促进根系生长调节水稻养分吸收；另一方面通过调节水稻氮吸收相关基因的表达增强对氮的获取[25−26]。

1.4 甘蔗联合固氮

甘蔗是高效的联合固氮作物。在低氮肥输入条件下，接种具有固氮能力的内生菌，不仅可以直接为甘蔗提供氮素[27]，还能改善甘蔗氮代谢、激素信号转导、生长素合成等功能[28−29]，提升对其他来源氮素的转化利用。例如，甘蔗接种内生固氮菌 (*Klebsiella variícola*) 共同培养后，甘蔗胺氧化酶、抗氧化酶、植物激素均显著增加，固氮菌通过刺激多胺代谢途径和植物激素产生途径促进甘蔗生长[29]。在低氮条件下，甘蔗内生固氮菌 (*Enterobacter roggenkampii* ED5) 增加了叶片中谷氨酰胺合成酶 (GS) 和 NADH-谷氨酸脱氢酶 (NADH-GDH) 基因表达量，将固氮酶产生的铵通过 GS 催化途径或 NADH-GDH 途径转化为谷氨酰胺，将固氮菌产生的氮不断用于自身光合产物的同化积累[30−31]。对甘蔗内生固氮菌 (*Pseudomonas aeruginosa* DJ06) 的全基因组分析表明，该固氮菌存在固氮、氨同化、铁载体、生长素、磷酸盐代谢、生物膜等多个重要的植物促生基因，接种 DJ06 后甘蔗生长素含量比未接种处理提高 37.38%，有效促进了植物发育[32]。

2 禾本科作物联合固氮关键部位

禾本科作物的联合固氮菌广泛存在于根际、根内和地上部，在这些关键部位，固氮微生物可从大气中捕捉氮气并转化为植物可利用的形式，通过与根际、根内和地上部微生物的复杂相互作用，提升

禾本科作物的氮吸收利用效率。

2.1　根际生物固氮

根际固氮菌对根系分泌物的趋化作用是禾本科作物根际生物固氮的关键机制 (图 1)。研究证实，在氮胁迫条件下植物通过根系分泌物诱导调控参与氮循环微生物的活性，从而增强作物对氮素的获取[64]。例如，类黄酮柚皮素 (flavonoid naringenin) 可显著促进巴西固氮螺菌 (*Azospirillum brasilense*) 在小麦根际定殖[65]。在墨西哥土壤贫瘠的 Sierra Mixe 地区，当地玉米品种可通过气生根分泌大量黏液招募固氮细菌，为植物提供 29%～82% 的氮源[66]，这些含有丰富海藻糖、半乳糖、阿拉伯糖等多糖的黏液为固氮菌营造了最佳生态条件，不仅满足其能量需求，还支持固氮酶的微氧新陈代谢和必要的定殖机制。这种气生根粘液与固氮菌的关系也在蔓性野牡丹 (*Heterotis rotundifolia*) 中被发现[67]。在小麦、大麦和高粱等作物根系同样发现了类似的黏液物质[68−71]。Bennett 等[72]推测，玉米根系黏液与固氮微生物之间的互作机制可能在其他作物中也普遍存在，这可能是植物根系生物固氮的共同特征。

根际“生物被膜形成”也是固氮菌适应外界复杂生态环境的一种策略[73]。生物被膜是由蛋白质、胞外 DNA、多糖等细菌分泌物组成的胞外聚合物，对于固氮菌在根部定殖至关重要。研究表明，在缺氮条件下水稻根际固氮菌 (*Pseudomonas stutzeri* A1501) 利用胞外多糖形成细胞囊结构 (cyst-like cells)，作为氧气扩散屏障，实现在自然有氧环境中生物固氮[74]。Yan 等[8]更是利用 CRISPR 基因编辑调控水稻中黄酮生物合成途径，通过增加芹菜素等化合物分泌来刺激固氮菌生物被膜形成，促进细菌在水稻根部定殖，这一策略在土壤氮素胁迫条件下，增强了水稻的氮素获取能力。

2.2　根内生物固氮

内生固氮菌的发现可能为非豆科作物开辟了一条“体内高效固氮”的新途径。与根际相比，宿主内生环境不仅可满足固氮所需的能量和低氧分压，还能够有效避免激烈的土壤微生物竞争和矿质氮对生物固氮的抑制作用[75−77]。植物内生菌的侵入主要是从

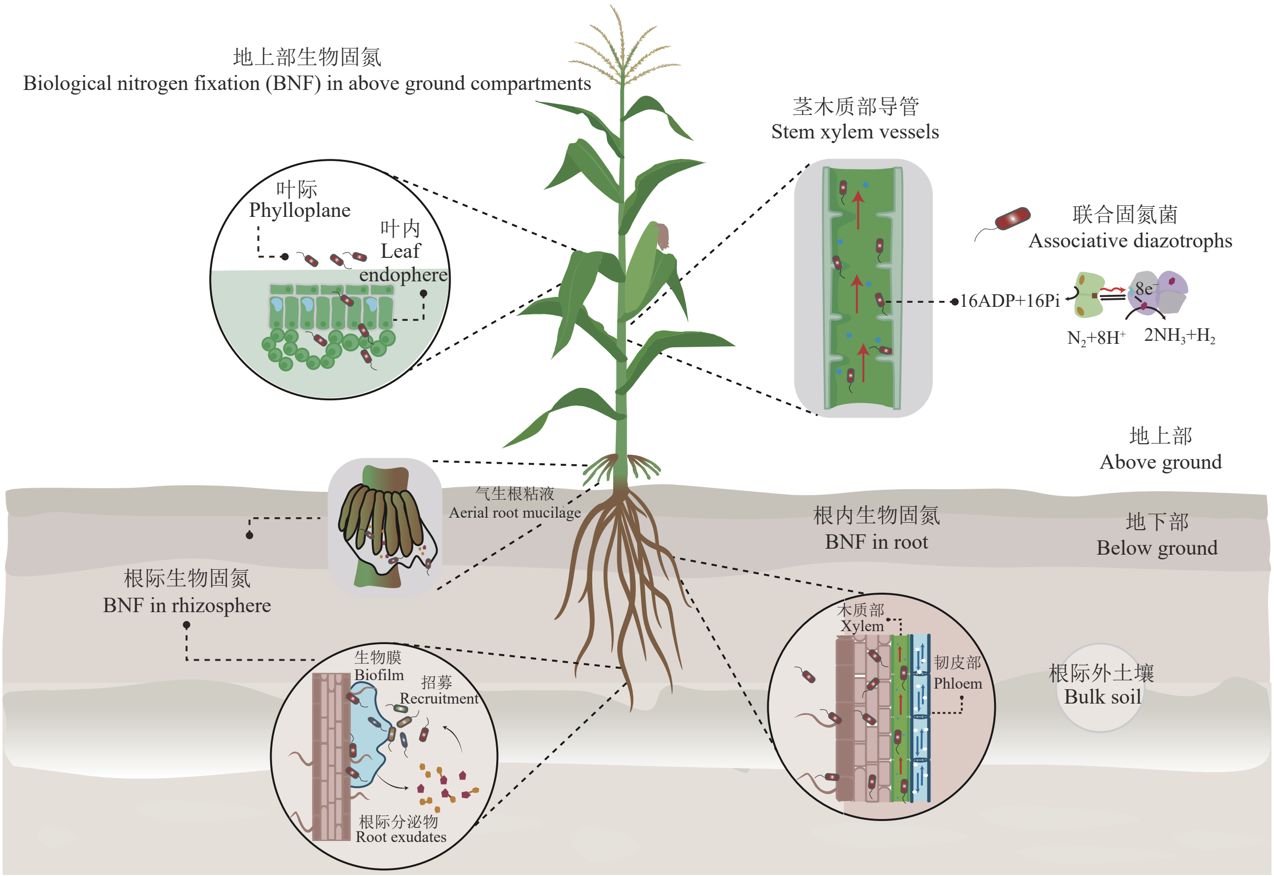

图 1　禾本科作物联合固氮关键部位

Fig. 1　Plant compartments of associative nitrogen fixation in gramineous crops

根毛或侧根的发生部位开始，根内细胞、叶肉、叶薄壁组织和木质部导管是内生固氮菌侵入后定殖最多的部位。James 等[78]利用 GUS (β-glucoronidase) 标记方法研究固氮菌 (*Herbaspirillum seropedicae* Z67) 的内生定殖过程，发现在初始阶段 GUS 染色在胚芽鞘、侧根以及主根和侧根的交界处最为强烈，该菌从侧根出芽处的裂缝进入根内，然后在根内细胞间隙定殖。Cocking 等[76]研究表明内生固氮菌 (*Gluconacetobacter diazotrophicus*) 可在根内细胞质定殖，且根内细胞条件适合固氮酶基因的表达。

另有研究发现，可以进入植物组织内并存活的细菌已经进化出独特的性状。例如，内生固氮菌 (*Klebsiella pneumoniae* 342) 能够在玉米、小麦等作物内部大量定殖，全基因组分析发现，该菌不仅含有参与趋化作用、鞭毛和纤毛形成的基因，还包括识别和降解植物源多糖的基因，表明该菌有极强的植物共生偏好性[79]。内生固氮菌 (*Azoarcus* sp. BH72) 也被证明存在编码纤维素酶和多聚半乳糖酶等细胞壁降解酶基因 (cell-wall degrading enzymes, CWDEs)[80]。此外，在内生菌定殖初级阶段，植物防御反应被激活性，细胞内释放的活性氧 (reactive oxygen species, ROS) 导致内生菌受到渗透胁迫。Alquéres 等[81]的研究指出，在固氮菌 (*G. diazotrophicus* PAL5) 的内生定殖过程中，超氧化物歧化酶和谷胱甘肽还原酶等 ROS 解毒基因转录水平显著上调，表明菌株 PAL5 的 ROS 清除酶系统在其水稻内生定殖中发挥重要作用。

2.3 地上部生物固氮

禾本科作物茎维管束为内生固氮菌提供了潜在的生存空间。研究报道，木质部导管的腔内定殖是内生细菌传播到其他植物营养器官的重要途径[82]。木质部单元间穿孔板的孔隙大小可以确保细菌的顺利通过[83]。研究表明，固氮菌 (*H. seropedicae* Z67) 定殖根系细胞间隙后，部分细菌可穿透中柱鞘，进入到根系木质部导管，最后定殖于茎和叶片的表皮细胞间隙和植物气孔下腔[78]。Zhang 等[3]通过比较玉米各部位微生物群落进一步发现，茎木质部伤流液中参与氮循环的微生物数量明显高于其他部位，在长期不施肥条件下茎木质部固氮酶基因 (*nifH*) 占 16S rRNA 总拷贝数的比例是根内的 2 倍和叶内的 4 倍，表明低氮胁迫下茎木质部富集了更高比例的固氮菌。

植物叶际包括附生层 (episphere) 和内生层 (endosphere)，同样为固氮菌发挥固氮功能提供了重要场所[84]。对玉米和水稻叶际固氮微生物群落的研究发现，肠杆菌属 (*Enterobacter*) 在玉米叶际中高度富集[33]，不动杆菌属 (*Acinetobacter*) 在水稻叶际中占主导地位[85]。此外，叶际附生层固氮微生物群落 α 多样性显著高于内生层。变形菌门 (Proteobacteria) 在叶际附生层和内生层皆为最主要的优势类群 (>90%)，而厚壁菌门 (Firmicutes) 在附生层中更为丰富[84]。尽管叶际环境相对苛刻，营养物质相对贫乏，但某些决定微生物在根际或内层定殖的关键因素，如胞外多糖、鞭毛、生物表面活性、自由基解毒蛋白、多功能代谢和群体感应信号分子等，在叶际定殖中也发挥重要作用[86]

3 禾本科作物生物固氮调控途径

3.1 宿主作物对固氮微生物的调控作用

作物–微生物共生关系的建立是一个复杂的过程 (图 2)。与豆科作物共生固氮相比，联合固氮菌与宿主之间的关系更加松散，但它仍然对植物基因型存在偏好性。宿主植物通过调控相应基因表达，并释放氨基酸、香豆素、有机酸和类黄酮等代谢产物，与固氮微生物建立联系[87−89]。Yin 等[90]研究发现，与栽培水稻相比，野生稻根际环境显著富集了甲基杆菌属 (*Methylobacterium*)、鞘氨醇单胞菌属 (*Sphingomonas*) 等特定细菌，其中甲基杆菌属固氮菌是野生稻最主要的富集类群，表明不同基因型水稻对根际固氮菌具有主动选择作用。研究表明，在养分胁迫条件下，宿主基因型对微生物群落结构的影响更为直接[91]，植物通过遗传因子整合胁迫信号[92]，倾向于基于功能需求为原则招募更多有益于自身氮素获取的相关功能微生物[93]。例如，在小麦中，黄酮类柚皮素和大豆苷可以促进内生固氮菌 (*Azorhizobium caulinodans* ORS571) 定殖，通过向宿主植物提供固定氮源以缓解宿主氮胁迫[94]。目前，豆科植物与根瘤菌之间的共生互作调控途径、关键基因、信号物质已被大量报道[95]。然而，关于禾本科作物与固氮微生物之间的信号通路与调控机制尚不清楚。为深入理解这种联合固氮关系，有必要开展全基因组关联分析 (genome-wide association study, GWAS) 以厘清植物基因型、宿主表型和固氮微生物组三者之间的互作关系。

3.2 微生物之间的协同调控作用

微生物之间的相互作用在调控固氮微生物功能潜力方面发挥至关重要的作用，主要包括固氮菌与其它细菌互作、固氮菌与真菌互作和固氮菌与病毒

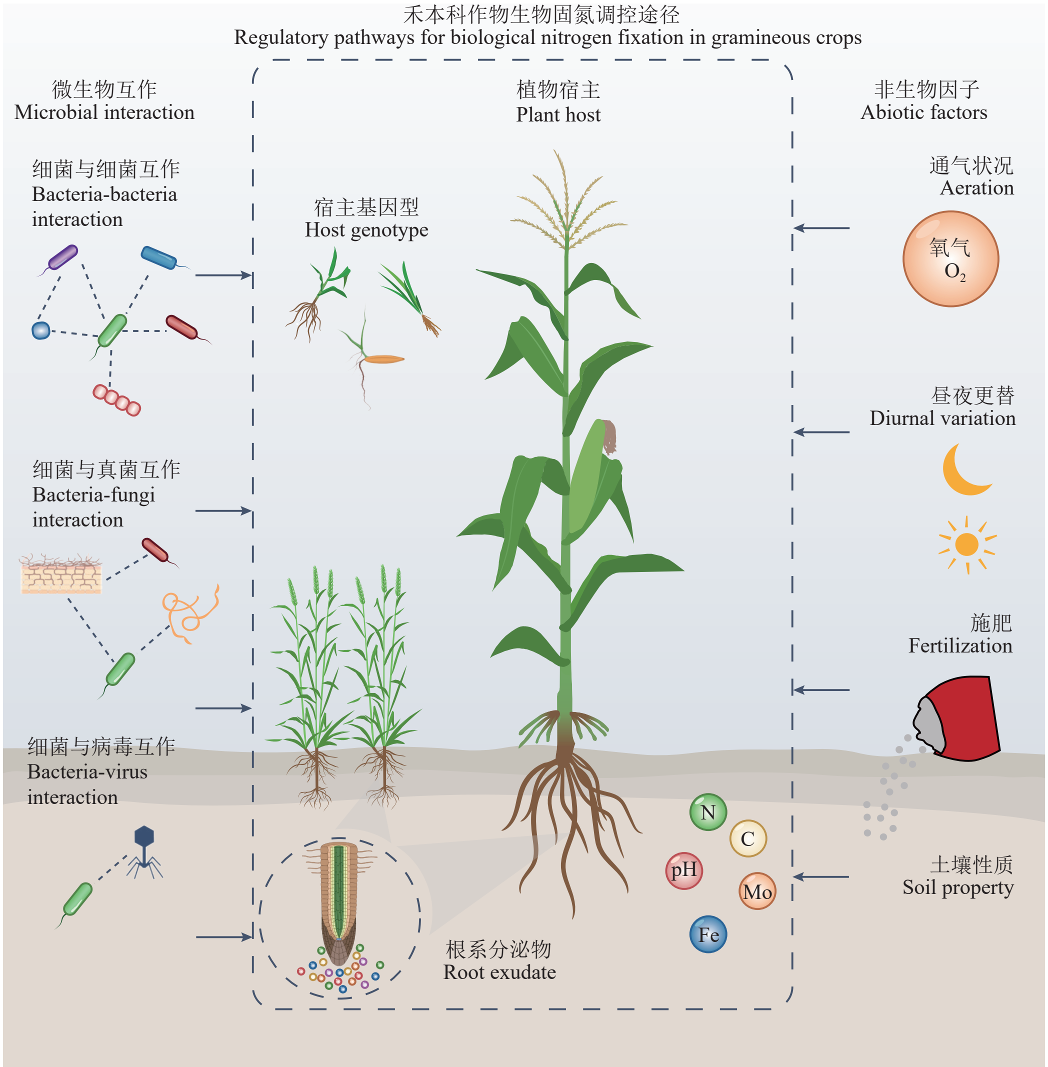

图 2　植物宿主、微生物互作和非生物因子对禾本科作物生物固氮的影响

Fig. 2　The effects of plant host, microbial interaction, and abiotic factors on biological nitrogen fixation in gramineous crops

互作。Zhang 等[3]从不同气候种植区玉米茎木质部伤流液中分离得到一组稳定存在的核心微生物，其中包括 2 株固氮细菌和 12 株非固氮细菌，研究发现这些非固氮菌均不同程度上促进了两株固氮菌的固氮酶活性，全基因组分析表明这些非固氮菌可能通过调控木质部微环境中氮和氧浓度来协助固氮菌进行固氮。最新的研究表明植物气生根粘液中固氮菌和真菌之间存在互作关系。Pang 等[67]研究发现，蔓性野牡丹 (*Heterotis rotundifolia*) 气生根粘液招募大量固氮菌的同时也招募了部分真菌，这些真菌具有广泛的抗菌活性，能够抑制超过 100 种病原微生物，但选择性地促进固氮微生物生长，确保了固氮功能的持续发挥。多项基于微生物网络分析的研究表明，丛枝菌根真菌和固氮菌之间的合作提高了固氮效率[96–97]。在豆科作物中，丛枝菌根真菌通过分泌脂壳寡糖 (LCOs) 和低壳寡糖 (COs) 等信号分子来激活丛枝菌根真菌和豆科作物之间的互作；同时，这些信号分子驱动豆科作物与固氮细菌之间相互作用并促进根瘤形成[98–99]。最近的研究表明，低养分条件下水稻和大麦等禾本科作物对 LCOs 和 COs 同样存在感知反应[100]；然而，真菌及其分泌的信号分子影响禾本科作物联合固氮的机制还需深入探索。病毒群落能够调控微生物群落并携带大量的辅助代谢基因，是碳氮循环的重要参与者[101–102]。例如，Kolan 等[103]探究了固氮蓝细菌 (*Cyanobacteria*) 与其噬菌体之间的互作，与野生型蓝细菌相比，17 株具有噬菌体耐受性的蓝细菌在缺氮条件下存在明显的营养缺陷，表明噬菌体抗性限制了蓝细菌固定氮的能力。目前，关于病毒与固氮菌互作及其影响禾本科作物联合固氮的研究还相对匮乏。

3.3　非生物因子对固氮微生物的调控作用

氧气和氮素对固氮酶的抑制作用以及高能耗是

限制联合固氮效率的 3 个关键因素。固氮酶对氧气高度敏感，微氧环境下才能保持正常活性[104]。Li 等[105]在中国东部 10 块旱地和水田开展^{15}N 标记试验后发现，水田土壤固氮效率显著高于旱地，间歇性灌溉和长期淹水引起的氧气浓度变化可能是这种差异的重要诱因。生物固氮是一个高耗能过程，同化一分子氮气，需要消耗 8 个高能电子和 16 个 ATP，而禾本科作物与固氮菌之间松散的共生关系并不能像豆科作物根瘤那样持续为固氮菌提供能量供应。因此，联合固氮菌更愿意直接从土壤中吸收氮养分，这也是禾本科作物固氮效率不高的重要原因。一般而言，农田生态系统长期施加无机氮肥将抑制固氮菌的固氮功能[106]，降低微生物群落固氮基因丰度和多样性[107−109]。在小麦−大豆轮作农田土壤中施加 35 年无机氮肥后，Fan 等[107]发现土壤生物固氮效率降低 50%，这种功能损失与地杆菌属 (*Geobacter*) 为主的固氮菌丰度下降有关。5 年连续施加氮肥也导致玉米地土壤 *nifH* 基因丰度降低 53.7%～79.7%[108]。相反，多项研究表明施加粪肥、生物炭和秸秆等有机肥通常能够增加生物固氮效率，这可能与施加有机肥改变土壤有机碳含量、土壤铁矿物含量、土壤 pH 和微生物群落结构等有关[109−110]。全球荟萃分析表明，氮素对生物固氮的抑制作用随着土壤有机碳含量增加而减弱[111]。Yu 等[110]发现长期施用有机肥显著增加了土壤短程有序 (short-range-ordered, SRO) 铁矿物含量，导致土壤中总钼含量提高近 30%，钼有效性的增加进一步导致土壤 *nifH* 基因丰度和固氮酶活性分别增加 14% 和 60%。此外，添加有机肥还可以缓解低土壤 pH 值对固氮酶活性的抑制作用[109]。最新研究表明，昼夜更替和温度对生物固氮也有显著影响。Tang 等[112]构建的大肠杆菌 (*Escherichia coli*) 和克雷伯氏菌 (*Klebsiella oxytoca*) 等固氮突变菌株在夜晚 23℃ 时，将联合固定的氮素分泌到体外供植物吸收，在白天 30℃ 时不向胞外分泌氮素，而是将固定的氮素用于自身生长和增殖，这种昼夜温差变化下的间歇供氮为禾本科玉米提供了更多氮素。

4 禾本科作物秸秆分解与生物固氮

4.1 秸秆分解与生物固氮的潜在互补关系

据估算，全球每年玉米、小麦、水稻、甘蔗 4 大类作物秸秆产量达到 30 亿 t，占主要农作物秸秆量的 70%，截止 2021 年我国秸秆量已超过 9 亿 t[113]。一半以上的禾本科作物秸秆以直接还田方式实现循环再利用[114]。然而，禾本科作物成熟后主要将氮素运送至籽粒，导致其秸秆碳氮比较高。一般而言，禾本科作物秸秆碳氮比为 60～80，远高于土壤微生物生长最适碳氮比 20～30，导致秸秆还田后氮营养缺乏，秸秆分解缓慢[115]。相反，固氮微生物十分偏好这种低氮环境，如果通过生物固氮的方式输入氮源，可有效缓解秸秆还田后的氮素限制。此外，氮素供应不足还会造成秸秆还田后微生物分解者呼吸作用增强[116]，所形成的土壤局部微氧环境为高效固氮创造了条件。与此同时，秸秆分解产生的大量碳水化合物为生物固氮所需的高强度能量供应提供了保障。当前秸秆还田配施氮肥是主要农艺措施。因此，挖掘生物固氮在禾本科作物秸秆分解过程的氮素供应潜力，可有效减少农业化学氮肥的投入 (图 3)。

4.2 秸秆分解相关固氮微生物类群与分布

已经证实自然界存在大量的纤维素分解与生物固氮协同微生物。在凋落物分解过程中的土壤微生物基因组研究中发现，约 3/4 含有固氮基因的菌株也同时含有编码纤维素酶基因[117]，这也表明自然界微生物之间碳氮代谢物的交叉取食机率非常高[118−119]。1983 年 Waterbury 等[120]第一次从腐木船虫的腺体中分离出具有纤维素分解和生物固氮的多功能菌株。此后在切叶蚁巢的碎叶分解菌圃中发现丰富的泛菌、克雷伯氏菌等固氮菌，为真菌分解树叶提供氮源[121]。我国江苏水稻土中也分离出 1 株克雷伯菌 (*Klebsiella* sp. C-3)，同时具有秸秆分解和固氮功能[122]。目前在土壤和海洋中，已发现属于不同菌属的细菌能同时产生纤维素酶和固氮酶，如芽孢杆菌属 (*Bacillus*)、梭菌属 (*Clostridium*)、固氮菌属 (*Azotobacter*)、热酸菌属 (*Acidothermus*) 等[123−127]，并将其命名为纤维素分解固氮菌 (cellulolytic nitrogen-fixing bacteria, CNFB)[128−129]，成为当前探索土壤微生物多功能的前沿热点。目前研究发现，对添加秸秆敏感的固氮菌主要集中于慢生根瘤菌属 (*Bradyrhizobium*)、固氮螺菌属 (*Azospirillum*)、厌氧粘细菌属 (*Anaeromyxobacter*)、伯克氏菌属 (*Burkholderia*)、克雷伯氏菌属 (*Klebsiella*) 和伯克霍尔德菌属 (*Paraburkholderia*)[130−131]，其中，施用秸秆使慢生根瘤菌属和固氮螺菌属等丰度显著升高，并强化了它们之间的互作关系[132−133]。

4.3 影响秸秆分解中生物固氮的关键因素

秸秆还田引起的土壤 C/N 升高是影响秸秆分解过程中固氮功能的关键因素[134−137]。在氮限制下固氮菌活性普遍较高[138−139]；相反，大量施用氮肥土壤

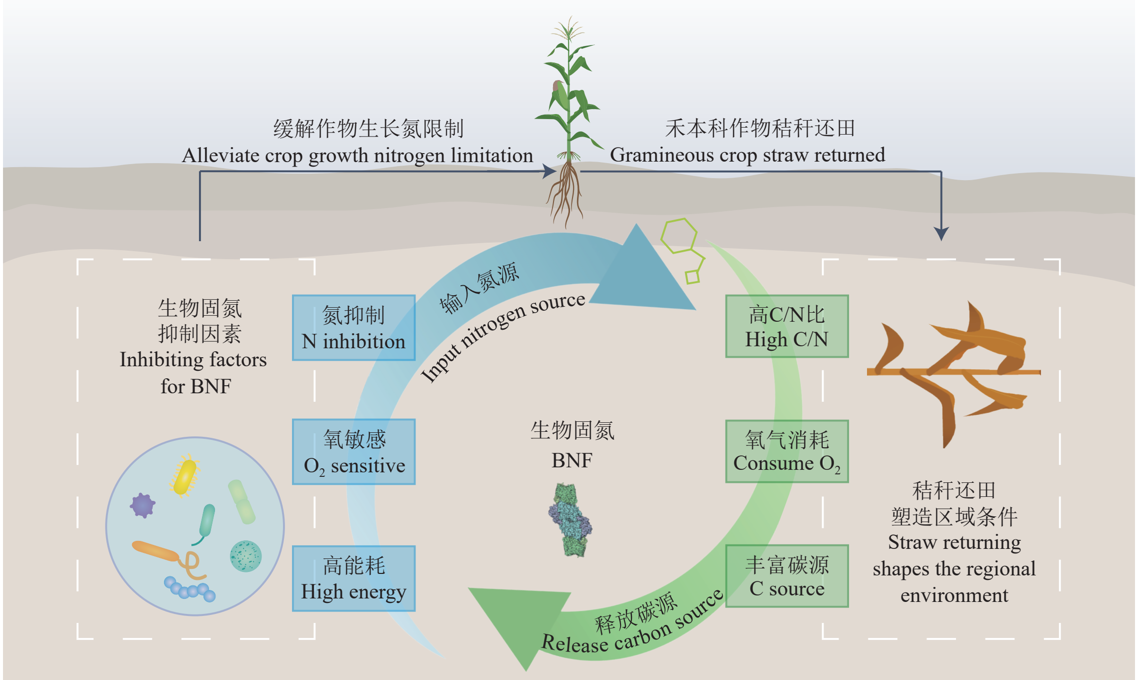

图 3　秸秆分解与生物固氮的潜在互补关系

Fig. 3　Potential complementary relationship between straw decomposition and biological nitrogen fixation (BNF)

nifH 基因丰度和固氮能力下降[140–141]。研究表明，土壤速效钾含量也与秸秆分解过程中固氮菌的组成有关[133]。钼元素是固氮酶的重要组分，由于秸秆中钼含量较低，施用一定量钼肥可提高秸秆分解中生物固氮能力[142]。秸秆分解中固氮菌对环境因子变化敏感，其功能主要由氧气浓度、土壤温度和光照等环境因素共同调控。例如，随着温度的升高，秸秆分解相关酶和固氮酶活性均显著增加，进而促进秸秆分解[133, 143]。研究发现，高温地区作物秸秆分解率和固氮量均显著高于寒冷地区[130, 144]。此外，在水田环境中，蓝藻是重要的光能自养型固氮菌，但秸秆还田影响光照条件，进而对固氮效率产生影响[145]。

5　生物固氮多功能合成菌群构建与应用

固氮菌剂的研发和应用是替代化学氮肥、促进农业高质量发展的有效策略。近年来，人们越来越关注利用合成菌群 (synthetic community) 来促进植物生长[146–147]。与单一微生物菌剂相比，合成菌群能够减轻单个菌株的代谢负担，提高生态功能的整体执行效率[148]，增强抵御环境波动的能力[149]。

目前，包括生物固氮功能在内的多功能合成菌群已经被构建并应用于多种禾本科作物的促生[3, 150–151]和生物防治[150]等方面。例如，Zhang 等[3]通过自上而下策略和交叉对比，从玉米茎木质部伤流液中获得 14 个核心细菌分类群，并建立了由两个核心固氮菌和两个协助菌组成的高效固氮合成菌群，利用绿色荧光蛋白 (green fluorescent protein, GFP) 标记菌株和 ^{15}N 同位素稀释方法证实该高效固氮合成菌群能够为玉米茎提供 11.8% 的总氮。Jiang 等[151]利用纯培养技术和生物信息学交叉验证，获得 21 株在 3 种土壤类型下均存在的玉米根际细菌类群，含有固氮、解磷、产植物生长素 (IAA) 等促进植物生长的微生物，由此构建的合成菌群使玉米低养分条件下根茎鲜重比增加 78%～121%。类似地，Liu 等[150]从小麦根际土壤中分离出 40 株细菌，选取 8 株具有固氮、解磷、产 IAA、抗病等 4 种功能菌株构建的合成菌群，使得小麦植株根系生物量、地上部生物量和成活率均显著高于未接种合成菌群处理。除了植物促生功能，固氮微生物也被纳入有机质或有机污染物降解相关的合成菌群。例如，Zhao 等[152]利用自下而上的方法构建了包含微杆菌 (*Microbacterium* sp. H2)、链霉菌 (*Streptomyces werraensis* F3) 和芽孢杆菌 (*Bacillus amyloliquefaciens* JF-1) 的合成菌群 HY-1，进一步将合成菌群 HY-1 应用于秸秆还田土壤中发现，与单菌相比，合成菌群增加了固氮酶和纤维素降解酶的活性，使得玉米秸秆腐解速率提高 37.91%，并显著促进了玉米幼苗的生长。Wang 等[153]构建了包含 1 种固氮菌 (*Azotobacter chroococcum* HN) 和 1 种

芘降解菌 (*Paracoccus aminovorans* HPD-2) 的合成菌群，在缺氮环境中固氮菌向芘降解菌提供了氮养分，促进了污染物芘降解。

6 禾本科作物生物固氮展望

小麦、玉米、水稻、甘蔗等禾本科作物是全球最主要的农作物，种植面积广泛，尽管单位固氮效率低于豆科作物，但是联合固氮过程普遍存在，所以禾本科作物固氮潜力同样巨大。近年来，在禾本科作物根、茎、叶中均发现大量不同类型固氮菌，但宿主作物与联合固氮菌之间是一种较为松散的互作关系，使得禾本科作物生物固氮田间应用未能达到理想效果。增强并优化这种联合固氮关系对于充分发挥禾本科作物的固氮潜力至关重要。当前，禾本科作物生物固氮研究仍面临诸多挑战，包括固氮菌类群和功能尚未充分挖掘，基因层面认识不足；固氮与其他功能细菌、真菌和病毒之间的作用关系理解有限，协同增效机理不清；固氮菌在作物根际、根内和地上部植物器官的定殖过程尚不清楚，宿主基因型以及非生物因素影响下的菌−植互作机制尚不明确。因此，针对上述问题，未来禾本科作物生物固氮研究应聚焦于以下几个方面：

1) 高效固氮微生物类群和功能挖掘。重点利用高通量培养组学、宏基因组学以及单细胞拉曼光谱等新的技术方法筛选和表征复杂环境中的关键固氮类群，尤其是深入挖掘地上部内生固氮菌功能潜力，结合基因组尺度代谢模型解析固氮微生物的遗传和代谢特征，为固氮菌种资源的利用和生物工程改造提供基础。

2) 固氮菌与其他微生物互作关系及协同增效机制解析。重点阐明固氮菌与秸秆分解菌等互补功能的细菌、真菌和病毒之间的协同增效机制，综合利用“自上而下”和“自下而上”策略，构建群落稳定、功能多样、效果显著的合成微生物群落，为禾本科作物联合固氮在农业生产中高效应用提供强有力的技术支撑。

3) 环境−作物−固氮微生物互作与优化调控。重点解析不同气候条件、土壤类型以及田间管理措施等条件下作物−微生物信号感知、微生物定殖以及与作物互作增效的分子机制，利用基因编辑 (如 CRISPR) 等现代分子生物技术手段，靶向调控生物固氮过程，定制并优化禾本科作物的微生物共生环境，旨在提升禾本科作物的生物固氮能力，为推动农业高质量发展提供重要支持。

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